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BASIS FOR REQUESTING CORRECTION OF THE US EPA TOXICOLOGICAL REVIEW OF CHLOROPRENE

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ACRONYMS AND ABBREVIATIONS

ADAF	age-dependent adjustment factor
AIC	Akaike Information Criterion
BCME	bis(chloromethyl)ether
BMD	benchmark dose
BMD10	benchmark dose at the 10% extra risk level
BMDL	lower 95% confidence limit of the benchmark dose
BMDL10	lower 95% confidence limit of the benchmark dose at the 10% extra risk level
DAF	dosimetry adjustment factor
DPE	Denka Performance Elastomer, LLC
EDB:	ethylene dibromide
F1	first generation
IARC	International Agency for Research on Cancer
IRIS	Integrated Risk Information System
IUR	inhalation unit risk
LOAEL	lowest-observed-adverse-effect level
$\mu g/m^3$	microgram(s) per cubic meter
MOA	mode of action
NATA	National Air Toxics Assessment
NDMA	nitrosodimethylamine
NOAEL	no-observed-adverse-effect level
NRC	National Research Council
NTP	National Toxicology Program
PBPK	physiologically based pharmacokinetic (model)
POD	point of departure
ppm	parts per million
Ramboll Environ	Ramboll Environ US Corporation
RR	relative risk
SIR	standardized incidence ratio
SMR	standardized mortality ratio
US EPA	United States Environmental Protection Agency
VCM	vinyl chloride monomer
WHO	World Health Organization
WOE	weight of evidence

EXECUTIVE SUMMARY

Background

In 2010, the United States Environmental Protection Agency (US EPA) Integrated Risk Information System (IRIS) program published a review of the epidemiology and toxicology literature on chloroprene to provide scientific support and rationale for hazard and dose-response assessment in IRIS, including deriving an inhalation unit risk (IUR) and other values for chronic exposure (www.epa.gov/iris).

In the "Toxicological Review of Chloroprene" (hereafter referred to as the "2010 Review") (US EPA 2010a), US EPA concluded that chloroprene was "likely to be carcinogenic to humans" based on (1) statistically significant and dose-related information from an National Toxicology Program (NTP 1998) chronic inhalation bioassay demonstrating the early appearance of tumors, development of malignant tumors, and the occurrence of multiple tumors within and across animal species; (2) evidence of an association between liver cancer risk and occupational exposure to chloroprene; (3) suggestive evidence of an association between lung cancer risk and occupational exposure; (4) the proposed mutagenic mode of action (MOA); and (5) structural similarities between chloropren e and known human carcinogens butadiene and vinyl chloride (US EPA 2010a).

The 2010 Review d erived an IUR for lifetime exposure to chloroprene of 5×10^{-4} per microgram per cubic meter ($\mu g/m^3$). This is the 5th highest IUR generated by US EPA to date for any chemical (not including carcinogenic metals or coke oven emissions) classified by US EPA or the International Agency for Research on Cancer (IARC) as a known or likely/probable human carcinogen. As outlined in detail below, we have determined that US EPA's classification relied on questionable , non-transparent evaluation and interpretation of the toxicological and epidemiological evidence. Therefore, the IUR for chloroprene was not based on the best standard methods US EPA has used for other carcinogens.

The IRIS Process: Challenges, Recent Changes, and Recommendations for Improvement

The US EPA IRIS process has been subject to high-level constructive criticism. Most noteworthy, subsequent to the 2010 Review, the National Research Council (NRC) of the National Academies of Science (NAS) published a series of reports recommend ing important changes to improve the IRIS process (NRC 2011, 2014). The recommendations were well received by US EPA, but have not yet been fully implemented, and have not been applied to previously published reviews. In particular, NRC (2011, 2014) emphasized the importance of transparency and rigor in the review methods. NRC (2011) provided guidance on development of inclusion and exclusion criteria for studies , and on methods for evaluating and taking into account various forms of bias and other methodologic characteristics that could impact study findings.

While the 2010 Review meets some of these NRC recommendations , it does not meet other key standards such as the evaluation and synthesis of the epidemiological and mechanistic data , and would benefit from their consideration and application. A transparent evaluation and integration of the published

epidemiological and toxicological evidence on chloroprene carcinogenicity highlights the need to reconsider US EPA's classification of chloroprene as "likely to be carcinogenic to humans" to be in line with the weight of evidence and the International Agency for Research on Cancer's (IARC 1999) classification of chloroprene as "possibly carcinogenic."

Toxicological Evidence

US EPA should evaluate the animal toxicological data that form the basis of the estimated chloroprene inhalation unit risk (IUR) in accordance with the NRC recommendations and US EPA standard risk evaluation methodologies. US EPA relied on the animal studies conducted by the NTP that showed very little consistency across species in tumor incidence and sites. These results indicated substantial species differences and demonstrated a unique sensitivity in the female mouse, with lung tumors being the most sensitive endpoint. Thus, US EPA used the female mouse data to derive the IUR, but without fully accounting for important pharmacokinetic differences between the mouse and humans.

In addition to revisiting the reliance on the animal dataset for the estimation of the IUR, US EPA should critically re-evaluate and integrate the cytotoxic and genotoxic evidence for chloroprene. The evidence from these studies indicates that chloroprene acts through a different mode of action (MOA) than the structurally similar and known human carcinogen 1,3-butadiene. Based on an evaluation consistent with the NRC (2011, 2014) recommendations, chloroprene's genotoxicity profile lacks several attributes necessary to conclude that there is a mutagenic MOA. Instead, the evidence supports site-specific cytotoxicity as a more likely MOA, as opposed to US EPA's conclusion that chloroprene acts *via* a mutagenic MOA.

Epidemiological Evidence

It is also necessary to critically evaluate the available epidemiological evidence on occupational chloroprene exposure. US EPA evaluated the epidemiological evidence of chloroprene carcinogenicity based on several occupational cohorts from around the world. This evaluation, however, would have benefited from more transparency and rigor with regard to how individual study quality was assessed and weighted in the overall weight-of-the-evidence assessment. In particular, US EPA did not assign more weight to the most recent epidemiological study by Marsh *et al.* (2007a, b), which also is the largest and most robust study to date. This study has been rated by other scientists as the best quality study available in part because it has the most comprehensive characterization of chloroprene exposure (Bukowski *et al.* 2009). Instead, US EPA equally weighted this study with poorer quality Russian, Armenian, and Chinese studies.

Marsh *et al.* (2007a, b) reported no excess occurrence of lung or liver cancers among chloroprene exposed workers. In fact, overall and for all sub-cohorts defined by specific plant(s), standardized morality ratios (SMRs) based on local reference rates were all below 1.0, providing no indication of any excess of these cancers among chloroprene exposed workers. US EPA, however, discounted this primary finding, and instead interpreted a correlation between exposure level and risk relative to a comparison subgroup where the comparison group exhibited

anomalously fewer cancers than expected, creating the appearance of an increased risk in the higher exposure groups. Furthermore, US EPA overlooked that there were as few as two liver cancer deaths in the comparison subgroup, likely reflecting a random deficit among this group. The US EPA summary of this study indicates incomplete evaluation and misinterpretation of the published results. Properly interpreted, the evidence does not demonstrate an association between occupational chloroprene exposure and human cancer incidence.

US EPA's Derivation of the Chloroprene IUR

US EPA derived the current chloroprene IUR based on a number of assumptions that are not substantiated by the scientific evidence, contributing to overestimation of an already conservative risk estimate *(i.e.*, one based on the most sensitive species, gender, and endpoint). Specifically, US EPA based the chloroprene IUR on a composite estimate of risk based on multiple tumors observed primarily in mice, not just the lung tumors for which the data were more conclusive. US EPA then assumed that the female mouse-based IUR was representative of continuous human exposure, and that lung tumors were systemic rather than portal-of-entry effects; US EPA also rounded up at various stages of adjustment. Finally, US EPA applied an age-dependent adjustment factor (ADAF) based on insufficient data to support a mutagenic MOA.

A PBPK Model for Chloroprene

In calculating the IUR, US EPA should have used the available pharmacokinetic model for chloroprene. Himmelstein *et al*. (2004 a,b) developed a physiologically based pharmacokinetic (PBPK) model for chloroprene to help explain the divergent results observed across animal species. The model demonstrates why the mouse is the most sensitive species and why humans are likely to be comparatively much less sensitive to the effects of chloroprene exposure.

The hypothesis that differences in pharmacokinetics are determinants of the observed species differences has been demonstrated for other chemicals, including vinyl chloride. Thus, it is scientifically appropriate that US EPA employ PBPK models, which use the best available science to adjust for these differences, to derive IURs for all chemicals, such as chloroprene, for which data are available.

US EPA did not use the PBPK model developed by Himmelstein *et al*. (2004 a,b) to inform the chloroprene IUR because US EPA noted that the data required to validate the model had not been published. However, all of the quantitative data necessary to refine and verify the critical metabolic parameters for the existing peer-reviewed PBPK model for chloroprene were available at the time of the 2010 Review and could have been used. Since then, additional data have been published, and the findings validate the model (Thomas *et al.* 2013, Yang *et al*. 2012, Allen *et al*. 2014). In particular, Allen *et al.* (2014) derived an IUR based on PBPK results and the incidence of respiratory cancer that was 100 times lower than US EPA's value, using a method which integrates both the animal and human evidence. Importantly, the IUR reported by Allen *et al*. (2014) is consistent with IURs for similar compounds such as vinyl chloride and 1,3-butadiene, which have stronger and more consistent epidemiological evidence of human carcinogenicity than chloroprene.

Calculation of an Up dated Chloroprene IUR

We conducted an updated analysis by applying the results from validated PBPK models to arrive at an IUR that includes an understanding of interspecies pharmacokinetics. We applied standard US EPA methodology and conservative assumptions to estimate of the potential cancer effects of chloroprene. Our estimated IUR is 1.1×10^{-2} per ppm or 3.2×10^{-6} per µg/m^3, which is of the same order of magnitude as the IUR derived by Allen *et al*. (2014), and which better reflects the scientific understanding of potential chloroprene cancer effects in humans. These results are also consistent with the results from validated PBPK models and comparisons with other structurally relevant compounds such as vinyl chloride and 1,3-butadiene, both recognized as known human carcinogens.

There is little scientific support for each of US EPA's conservative assumptions and subsequent adjustments. Combining a fuller understanding of interspecies pharmacokinetic differences and validated PBPK models with the results from the strongest epidemiological data provides the scientific grounds for updating the 2010 IUR and calls into question the strength of the evidence to support a "likely to be carcinogenic to humans" classification . Similar adjustments should also be considered in estimating the chloroprene inhalation reference concentrations (RfC), as species- and strain-specific differences are noted . This will assure that policies and decisions resting on these toxicity values meet the test of sound science, transparent methods, and reproducible findings.

Conclusions

The IUR published in the 2010 Review requires correction . An updated IUR should be based on the best available methodology as well as a valid interpretation of the body of published evidence. Correction is critical given that the IUR published in the 2010 Review is being used by US EPA for enforcement actions.

1 INTRODUCTION

In December, 2015, the United States Environmental Protection Agency (US EPA) published the 2011 National Air Toxics Assessment (NATA), indicating a high off-site air pollution cancer risk from emissions of chloroprene from the Neoprene production facility in LaPlace, Louisiana. The previous month, on November 1, 2015, Denka Performance Elastomer, LLC (DPE), had acquired the LaPlace Neoprene production facility. The underlying NATA risk calculations combined estimated ambient chloroprene concentrations from air modeling analyses with the cancer inhalation unit risk (IUR) value derived by the US EPA Integrated Risk Information System (IRIS) and documented in the Toxicological Review of Chloroprene (hereafter referred to as the "2010 Review") (US EPA 2010a).

On behalf of DPE, Ramboll Environ US Corporation (Ramboll Environ) prepared this summary review of the US EPA toxicity assessment for chloroprene, focusing on a detailed review of US EPA's derivation of the cancer IUR reported in the 2010 Review (US EPA 2010a). US EPA's chloroprene risk assessment calculations are based on and directly proportional to US EPA's IUR for lifetime exposure to chloroprene of 5×10^{-4} per micrograms per cubic meter ($\mu g/m^3$). The chloroprene IUR is the 5th highest IUR generated to date for any substance classified by US EPA or the International Agency for Research on Cancer (IARC) as a known or likely/probable human carcinogen (not including carcinogenic metals or coke oven emissions). The chloroprene IUR is orders of magnitude higher than IURs derived by US EPA for substances, such as vinyl chloride, 1,3-butadiene, and benzene, that have been classified by US EPA as known human carcinogens.[1] In contrast, chloroprene has been classified as "likely to be carcinogenic to humans" based on a weight-of-evidence (WOE) assessment that included an animal inhalation study conducted by the National Toxicology Program (NTP 1998) and four (of nine) epidemiological studies reportedly indicating increased risks for liver cancer (US EPA 2010a). It was noted that these data were insufficient to classify chloroprene as a known human carcinogen. On the other hand, IARC classified chloroprene as "possibly carcinogenic to humans," based on the same evidence from experimental animal studies and similar epidemiological evidence concluded that the human evidence was inadequate (IARC 1999).

Since the 2010 Review (US EPA 2010a), the National Academies of Sciences National Research Council (NRC 2011, 2014) has recommended substantive improvements to the IRIS evaluation process, calling for greater transparency including improved methods for and documentation of scientific study selection, critical review of study quality and limitations, and the synthesis of findings across studies. This has provided much of the impetus for changes to the IRIS process. Improvements in the critical evaluation of epidemiological study quality and bias were noted as especially important, as statistical associations in epidemiological studies are only meaningful if supported by rigorous study design and data quality control. In addition, NRC noted the need for improved approaches to integrating evidence across diverse lines of investigation —including evidence from animal

[1] https://www.epa.gov/fera/dose-response-assessment-assessing-health-risks-associated-exposure-hazardous-air-pollutants

experiments, mechanistic investigations and epidemiological studies —in drawing conclusions regarding carcinogenicity and in deriving unit risk factors for cancer. NRC recommended better evidence integration that considers and weighs the entire body of scientific evidence, and that does not rely on select and unrepresentative findings (NRC 2011, 2014). Similarly, using formaldehyde as an example, NRC recommended improved use of evidence in risk assessments. NRC (2011) recommended using physiologically based pharmacokinetic (PBPK) models to quantify demonstrated differences in pharmacokinetics across species, and further recognized PBPK models as a tool to support extrapolations between species, thereby reducing the uncertainty in quantitative risk assessments (NRC 2014). These NRC recommendations remain highly relevant to the evaluation of chloroprene. In **Section 2**, we highlight key recommendations made by the NRC for improvements to the IRIS process that potentially impact the chloroprene evaluation.

Consistent with the NRC recommendations to improve the scientific quality and validity of the 2010 Review, US EPA needs to address significant uncertainties associated with the derivation of the IUR. These uncertainties pertain to the human relevance of the animal evidence, and whether or not various cancer types observed in animal experiments should be combined in estimating potential cancer risk to humans. Studies available both at the time of the 2010 Review, and published since, demonstrate clear and significant pharmacokinetic differences between humans and animals (Himmelstein *et al.* 2004a, b; Yang *et al.* 2012; Thomas *et al.* 2013; Allen *et al.* 2014). These differences must be considered in order to derive a scientifically valid human cancer unit risk for chloroprene based on animal studies. In **Section 3**, we discuss the uncertainties associated with toxicological evidence; and in **Section 4** we propose that the available mechanistic evidence supports a cytotoxic, rather than mutagenic, MOA for chloroprene.

In **Section 5**, we discuss US EPA's evaluation of the epidemiological data. US EPA did not fully or accurately summarize the findings from the Marsh *et al*. (2007a, b) study, which represents the largest and most comprehensive epidemiological study of chloroprene to date. Marsh *et al*. (2007a, b) reported no evidence of increased risks of liver and lung cancer with occupational chloroprene exposure; however, US EPA drew contrary conclusions from small subsets of the Marsh *et al*. (2007a, b) data.

In **Section 6**, we discuss the uncertainty associated with the evidence presented by US EPA to support a classification of "likely to be carcinogenic to humans," noting that the weight of evidence narrative is incomplete and the evidence is weaker than US EPA reports, and is more consistent with a "suggestive" classification.

In **Section 7**, we summarize the uncertainties associated with the US EPA derivation of the IUR, and in **Section 8**, we compare the IUR for chloroprene to other chemicals that have been classified by US EPA and IARC as known or probably human carcinogens. This comparison shows that the IUR for chloroprene is substantially out of line with the US EPA risk evaluation of chemicals that are known carcinogens.

In **Section 9**, we summarize new evidence that indicates that a PBPK model is the most valid and appropriate means of quantifying the large differences between animal and human responses to chloroprene exposure and in **Section 10**, we use PBPK results and standard US EPA methods endorsed by NRC to calculate an IUR for chloroprene. In **Section 11**, we use exposure data from the Marsh *et al.* (2007a, b) study to calculate the expected incidence of cancer among workers using the 2010 US EPA IUR and using PBPK-adjusted IURs as a "reality check" to demonstrate that the PBPK-adjusted IUR, but not the US EPA-derived IUR, is consistent with the epidemiological findings.

In **Section 12** we discuss the need to apply pharmacokinetic modeling in the derivation of the RfC, which also suffers from application of default methodology that does not properly account for the known pharmacokinetic differences across species, and species- and strain-specific differences in response.

Lastly in **Section 13**, we conclude that an updated and corrected IRIS assessment, and especially an updated IUR, are warranted and urgently needed. The new assessment should combine the most up-to-date scientific evidence regarding chloroprene toxicity and carcinogenicity with improved and more transparent methods for conducting toxicological and epidemiological reviews, in accordance with the NRC recommendations and guidance (NRC 2011, 2014). We are confident that the substantive and procedural reasons for updating the IRIS assessment for chloroprene, as detailed in this report, will result in a valid and scientifically appropriate IUR for chloroprene that is also consistent with the assessments for other substances including several known human carcinogens.

2 THE IRIS PROCESS: CHALLENGES, RECENT CHANGES, AND NRC RECOMMENDATIONS FOR IMPROVEMENT

2.1 Purpose of the IRIS program

The IRIS program was developed to be the primary source of toxicological information for federal, state, and international regulatory agencies for setting risk-based regulatory standards. It was intended to provide consistency among toxicological assessments within US EPA. IRIS assessments contain hazard evaluations (determinations of whether substances are capable of causing disease) and dose-response assessments (determinations of the levels at which such effects occur) for various chemicals, including cancer and non-cancer outcomes.

2.2 Challenges in the IRIS process

While most of the IRIS assessments have been straightforward and well documented, others have proved to be more complex and challenging, sometimes lacking transparency of methods. These problems have led to significant variability and uncertainty regarding the calculated estimates of hazard or risk of health effects in humans. As a consequence, the NRC has been called on multiple times to review some of the more challenging or ambiguous assessments, including those for formaldehyde, dioxin, and tetrachloroethylene.

In perhaps the most critical evaluation, the NRC (2011) reviewed the draft "Toxicological Review of Formaldehyde - Inhalation Assessment" (US EPA 2010c) and outlined several general recommendations for the IRIS process, as well as some specific aspects needing improvement. Subsequently, Congress held several hearings regarding the IRIS program. A House Report (112-151) that accompanied the Consolidated Appropriations Act of 2012 (Public Law 112-74)[2] specified that as part of the IRIS process, US EPA had to incorporate the recommendations of NRC in its IRIS "Toxicological Review of Formaldehyde" where appropriate, based on chemical-specific information and biological effects. Congress requested that NRC oversee this process to ensure US EPA implemented the changes. Congress also directed that NRC should make additional recommendations as needed to further improve the program. In 2014, NRC released a report on the IRIS process, which largely described the findings in its 2011 formaldehyde review as they relate more broadly to the IRIS process (NRC 2014). The final Toxicological Review of Formaldehyde has not yet been released.

Subsequently, US EPA published a report entitled "Integrated Risk Information System (IRIS) Program: Progress Report and Report to Congress" (US EPA 2015) in which US EPA assured Congress that progress toward improving the IRIS process and addressing the NRC recommendations was continuing.

NRC (2011, 2014) also emphasized the importance of a detailed protocol, including making the methods and the process of the review transparent. Increased transparency provides not only the opportunity for meaningful peer review, but also

[2] Pub. No. 112-74, Consolidated Appropriations Act, 2012 available at https://www.gpo.gov/fdsys/pkg/PLAW-112publ74/pdf/PLAW-112publ74.pdf

for other investigators to verify the methods and replicate findings. The protocol should specify how studies will be evaluated and weighted according to quality rather than on the basis of findings; explicitly state the inclusion and exclusion criteria for studies; describe how study quality will be evaluated; and outline methods for evaluating and taking into account various forms of bias and other methodologic characteristics of the studies that could impact their respective conclusions. The 2010 Review did not follow such a protocol.

Another key criticism that the NRC (2011) made specific to the IRIS assessment of formaldehyde, and more generally to the IRIS program as a whole, was that the IRIS process lacked an appropriate framework for systematic review and integration of all applicable lines of evidence. NRC (2011) cited the systematic review standards adopted by the Institute of Medicine (2011) as being appropriate for such an analysis.

2.3 Recommendations for improvement of the IRIS process in updating the 2010 Review

Because the 2010 Review predates the NRC critique, it would benefit from application of many of their recommendations. For example, clearer descriptions of how the epidemiological evidence was evaluated would provide greater transparency. Similarly, epidemiological evidence should be evaluated for study quality and assessed for potential bias, as some of the strongest epidemiological evidence was misinterpreted (*i.e.*, from the Marsh *et al.*, 2007a, b studies), and results from some weaker studies (from Russia, Armenia, and China) were given equal weight.

US EPA's Guidelines for Carcinogen Risk Assessment (US EPA 2005) established study quality criteria for the WOE evaluation and for identifying and justifying the use of specific epidemiological studies in assessing evidence of carcinogenicity, as follows:

- Clear objectives
- Proper selection and characterization of comparison groups (cohort and reference)
- Adequate characterization of exposure
- Sufficient duration of follow-up
- Valid ascertainment of causes of cancer morbidity and mortality
- Proper consideration of bias and confounding
- Adequate sample size to detect an effect
- Clear, well-documented and appropriate methods for data collection and analysis
- Adequate response (minimal loss to follow-up)
- Complete and clear documentation of results

These points were similarly outlined in the NRC critique of the IRIS process (NRC 2014).

Based on a critical review of the animal toxicology evidence, important differences in chloroprene toxicity have been demonstrated across species that are explained by differences in pharmacokinetics. In such circumstances PBPK models are required to adjust for these differences and have been applied by US EPA for other chemicals. Although a chloroprene-specific PBPK model was available at the time of the 2010 Review, US EPA did not use it. Since the release of the 2010 Review, additional data and a fully validated PBPK model have been peer-reviewed and published. By incorporating the highest quality epidemiological studies and the most recently published data on the pharmacokinetics of chloroprene metabolism, deriving a scientifically sound IUR for chloroprene is straightforward. As demonstrated below, an IUR derived using methods applied by US EPA and the scientifically highest quality data publically available will produce an IUR that is over 150 times lower than the IUR published in the 2010 Review.

3 TOXICOLOGICAL WEIGHT OF EVIDENCE: ANIMAL STUDIES

3.1 Guidelines for evaluating toxicological studies

US EPA set forth criteria for the evaluation of toxicological data in the "Guidelines for Carcinogen Risk Assessment" (US EPA 2005). These guidelines are largely consistent with the NRC recommendations for IRIS (NRC 2014). However, US EPA did not apply these risk assessment guidelines in the 2010 Review in its evaluation and determination of the weight of evidence (WOE) available from the animal, mechanistic, and epidemiological studies of chloroprene. In this section, we discuss the toxicological evidence available to evaluate whether it supports carcinogenicity of chloroprene in humans.

3.2 Animal studies show important pharmacokinetic differences across species

US EPA based the 2010 IRIS IUR estimate for chloroprene primarily on the findings of a two-year inhalation study conducted by the NTP (1998). The NTP (1998) study found statistically significant increases in tumor incidence at multiple sites in the B6C3F1 mice, including: all organs (hemangiomas and hemangiosarcomas), lung (bronchiolar/alveolar adenomas and carcinomas), forestomach, Harderian gland (adenomas and carcinomas), kidney (adenomas), skin, liver, and mammary glands. With increasing exposures, the tumors generally appeared earlier, and statistically significant pair-wise comparisons were reported with increasing exposure level. F344/N rats were less sensitive to chloroprene exposures than B6C3F1 mice.

US EPA also considered results from another large study conducted by Trochimowicz *et al.* (1998) in Wistar rats and Syrian hamsters that showed a large variability in the tumor incidence and sites across species. Trochimowicz *et al.* (1998) found that although tumors appeared across multiple sites in both rats and hamsters, there were no statistically significant increases at any particular site, no significant trends observed with increasing concentration, and tumor incidence in less than 20% of hamsters. These results showed that the Wistar rat and the hamster are less sensitive to the toxicity of chloroprene than B6C3F1 mice or F344/N rats.

The results of the NTP (1998) and Trochimowicz *et al.* (1998) studies indicated that the mouse is the most sensitive species to chloroprene among the species tested, based on the concentrations at which statistically significant increases in tumor incidence were observed, as well as the number of tumor sites. In the NTP (1998) study, the incidence of lung tumors was observed to be statistically significantly elevated at the lowest exposure tested (12.8 parts per million [ppm]) in both female and male mice. Statistically significantly increased lung tumor incidence was not observed in any other animal species that was evaluated, including male and female rats administered chloroprene at concentrations up to 80 ppm. For other tumor sites, there were some statistically significantly elevated results in B6C3F1 mice and F344/N rats, but primarily limited to the highest exposure levels (80 ppm). For example, the incidence of liver tumors in mice were only statistically significantly increased in female mice at the highest exposure concentration tested

(80 ppm). For these reasons, the 2010 Review noted that the differences in response observed between the NTP (1998) and Trochimowicz *et al*. (1998) studies may be due to species and/or strain differences.

Thus, across all tested species, the data demonstrated that mice are the species most sensitive to chloroprene exposure and that the incidence of lung tumors is the most sensitive endpoint in mice. The findings therefore are specific to mice and not generalizable across animal species. Given the differences in response in the mouse as compared to other laboratory species following chloroprene exposure, it is particularly important to evaluate the potential for differences in pharmacokinetics to better characterize and explain the cross-species differences, particularly in developing an IUR intended to be predictive of human risk.

3.3 Conclusions

US EPA derived a chloroprene human IUR based not only on the highest IUR, which corresponded with the lung tumors (the most sensitive endpoint) and female mice (the most sensitive species and gender), but also, as discussed below, US EPA then calculated a human composite IUR that was based on multiple tumor sites in the female mouse. Rats were considerably less sensitive to the carcinogenic effects of chloroprene and thus were not considered further in the dose-response analysis; however, the observed lower incidence of tumors in rats than mice indicates significant species differences that cannot be disregarded in the human carcinogenicity evaluation.

4 MECHANISTIC EVIDENCE: CHLOROPRENE MODE OF ACTION

4.1 Guidelines for evaluating mechanistic studies

As with the evaluation of animal data, US EPA did not apply the guidelines for evaluation of mechanistic weight of evidence set forth in the "Guidelines for Carcinogen Risk Assessment" (US EPA 2005) and the NRC recommendations for IRIS (NRC 2014). In this section, we discuss the mechanistic evidence available to evaluate whether it supports a mutagenic mode of action (MOA) for chloroprene.

4.2 Mechanistic evidence for cancer effects from chloroprene do not support a mutagenic MOA

A key determinant of understanding whether an agent is carcinogenic is to establish an MOA. In the 2010 Review, US EPA hypothesized that chloroprene "acts via a mutagenic MOA involving reactive epoxide metabolites formed at target sites or distributed systemically throughout the body." US EPA noted that "this hypothesized MOA is presumed to apply to all tumor types" (US EPA 2010a), suggesting some non-independent events would be needed for the development of all of the tumors observed. In formulating this hypothesis of a mutagenic MOA, the 2010 Review did not present a description of whether or how the available evidence was critically evaluated, weighted and integrated. This is inconsistent with US EPA (2005) guidelines which indicated that the purpose of the hazard assessment is to "construct a total analysis examining what the biological data reveal as a whole about carcinogenic effects and MOA of the agent, and their implications for human hazard and dose-response evaluation." These 2005 guidelines are also consistent with the new NRC (2014) recommendations for the need for integration of the evidence to support scientific conclusions.

In providing supporting evidence for a mutagenic MOA, the 2010 Review focused on *in vitro* studies (using different exposure systems) in bacteria, with less weight placed on the results from *in vitro* studies in mammalian cells and *in vivo* studies.[3] In particular, in assessing whether chloroprene has a mutagenic MOA, the 2010 Review gave little weight to the studies conducted by the NTP and others (Tice 1988, Tice *et al*. 1988, NTP 1998, Shelby 1990, Shelby and Witt 1995). This also is contrary to the recommendations of NRC (2014) regarding evidence integration. The NTP (1998) study that served as the basis of the US EPA IUR for chloroprene states, "chloroprene was not mutagenic in any of the tests performed by the NTP."

Furthermore, the majority of the conventional genetic toxicology studies relied on in the 2010 Review did not report positive results following administration of chloroprene. In drawing conclusions concerning the chloroprene MOA, US EPA should have acknowledged the flaws and methodological limitations in the studies on which it relied. When these studies and their limitations are considered, along with the predominantly negative *in vitro* and *in vivo* genotoxicity tests, there is little evidence for concluding that chloroprene is mutagenic or genotoxic (NTP 1998, Pagan 2007). Therefore, this evidence should not be used to support a

[3] *In vitro* mammalian and *in vivo* studies are generally considered to be more relevant to effects that might be observed in humans (*e.g.*, Wetmore *et al.* 2013).

classification of chloroprene as a "likely" human carcinogen and should not influence the derivation of the chloroprene IUR.

In summary, the hypothesized MOA was based on four major assumptions by US EPA (2010a):

1. There are similarities in the MOA for the known human carcinogen 1,3-butadiene, which involves metabolism to a reactive epoxide intermediate
2. Chloroprene forms DNA adducts via its epoxide metabolite
3. Chloroprene is a point mutagen *in vitro*
4. Chloroprene is a point mutagen *in vivo*

However, the integration of the currently available evidence for chloroprene support none of these assumptions. A discussion of why the available science is inconsistent with these assumptions is provided in the following sections.

4.2.1 The chloroprene mutagenic profile is distinct from that of 1,3-butadiene

US EPA assumed that chloroprene has a similar MOA to that of 1,3-butadiene, which is metabolized to epoxide intermediates and is a rodent carcinogen. While both compounds may be carcinogenic in rodents, evidence is available that shows that the mutagenic and clastogenic profiles of 1,3-butadiene are considerably different from the profile of chloroprene (Tice 1988, Tice *et al.* 1988). Unlike 1,3-butadiene, chloroprene does not induce effects when tested in standard *in vivo* genotoxicity screening studies in mammals (Table 4.1). Although the reactive metabolite of chloroprene (1-chloroethenyl) oxirane does induce mutations *in vitro* in bacterial strains (Himmelstein *et al.* 2001a), neither the administration of chloroprene nor the reactive epoxide metabolite was genotoxic or mutagenic in *in vitro* mammalian cells, including Chinese hamster V79 cells (Himmelstein *et al.* 2001a, Drevon and Kuroki 1979). Also, unlike 1,3-butadiene, chloroprene was not genotoxic when tested *in vivo* (Tice 1988, Tice *et al.* 1988, NTP 1998, Shelby 1990, Shelby and Witt 1995).

Table 4.1. Comparison of the Mutagenic Profiles of Chloroprene and 1,3-Butadiene

Chemical	In Vitro Ames	In Vivo (B6C3F1 mouse)a		
		CA	SCE	Micronuclei
1,3-Butadiene	+	+	+	+
Chloroprene	+/-	-	-	-

a Exposure was 10-12 days (6 hr/day) inhalation (Tice 1988)

These findings indicate that the reactive metabolites formed from chloroprene are effectively detoxified *in vivo* in the concentration ranges studied. This is an important difference between chloroprene and 1,3-butadiene. In addition, 1,3-butadiene appears to be an effective somatic cell genotoxin in mice (Tice 1988), whereas chloroprene was not genotoxic in *in vivo* assays (Tice 1988, Tice *et al.*

1988, Shelby 1990, Shelby and Witt 1995, NTP 1998). The only published chloroprene-related study showing positive chromosomal aberrations *in vivo* was a study cited by Sanotskii (1976); but as acknowledged in the 2010 Review, this study was technically deficient and conflicted with stronger and more recent studies conducted by NTP in mice (Shelby 1990, NTP 1998).

Two other major differences between these chemicals are evident from the experimental data. First, the *ras* profile in lung tumors in treated animals is considerably different for chloroprene and 1,3-butadiene (Sills *et al.* 1999). Secondly, the toxic effects and histopathology observed in chloroprene-treated F344 rats and B6C3F1 mice are substantially different from those seen in 1,3-butadiene exposed animals (Melnick *et al*. 1996). These differences in toxic effects and histopathology suggest that the carcinogenic MOA for 1,3-butadiene also is different from that of chloroprene.

Furthermore, even if we disregard the assumption that chloroprene acts *via* a similar MOA as 1,3-butadiene, the chloroprene IUR is more than an order of magnitude greater than that of 1,3-butadiene. This is inconsistent with the assumption that these compounds have a similar MOA, and is also inconsistent with US EPA's underlying assumptions regarding the carcinogenicity and the potency of chloroprene relative to 1,3-butadiene.

4.2.2 Evidence does not support the formation of DNA adducts by chloroprene metabolism to an epoxide intermediate in vitro

The 2010 Review assumed that the chloroprene epoxide metabolite (1-chloroethenyl)oxirane forms DNA adducts. There is little evidence that this occurs *in vivo*. Although *in vitro* studies suggest an interaction between this metabolite and DNA adducts, this effect has not been confirmed *in vivo*. In addition, the lack of any observed genotoxicity *in vivo* as described above (Tice 1988, Tice *et al*. 1988, NTP 1998, Shelby 1990, Shelby and Witt 1995) does not support an interaction between chloroprene and DNA *in vivo*.

4.2.3 Evidence does not support mutagenicity of chloroprene in vitro

The 2010 Review also assumed that chloroprene is a point mutagen *in vitro*. However, the results of the bacterial mutagenicity studies are equivocal, at best, and the findings from the Ames tests question the classification of chloroprene as a mutagen (NTP 1998, Pagan 2007). The results from two studies indicated that chloroprene was mutagenic in *Salmonella typhimurium* TA100 and/or TA1535, particularly with the addition of S9 mix, which incorporates the metabolism of chloroprene (Bartsch *et al*. 1979, Willems 1980). Two other studies failed to show any increase in TA1535 or TA100 revertants, as shown in Table 4.2. Chloroprene was not mutagenic in *S. typhimurium* strains TA98 or TA1537 (Zeiger *et al*. 1987). Because toxicity to the Salmonella cells was reported for all of the studies, one can assume there was adequate exposure to chloroprene and its metabolites or oxidative degradation products, although concentrations and composition verification were not performed.

Table 4.2. Ames Test Results for Chloroprene with TA1535 and/or TA100

Study	Method	Exposure	Response	
			With S9 mix	Without S9 mix
Bartsch *et al*. 1979	Desiccator [a]	4 hours	++	+
Westphal *et al*. 1994	Pre-inc[b]	2 hours	-	-
NTP 1998	Pre-inc[b]	20 minutes	-	-
Willems 1980	Desiccator [a]	24-48 hours	++	+

[a] Plates sealed in desiccator at 37° C with tops removed.

[b] Chemical added to sealed tubes and mixed at 37° C.

Toxicity results further appear to be dependent on the exposure methods and the form of chloroprene tested *(e.g.*, newly distilled or aged). Westphal *et al*. (1994) confirmed the importance of both vehicle and decomposition products in assessing the mutagenicity of chloroprene. For example, they showed that freshly distilled chloroprene was not mutagenic, but chloroprene aged for as little as two to three days at room temperature was mutagenic in *S. typhimurium* TA100. The mutagenicity increased linearly with the age of the distillate, probably due to the presence of decomposition products such as cyclic dimers (Westphal *et al.* 1994). Therefore, it is not possible to conclude from published data that chloroprene is a point mutagen in bacteria.

Chloroprene also does not appear to be mutagenic in mammalian cells. Drevon and Kuroki (1979) were not able to induce point mutations when chloroprene was tested in Chinese hamster V79 cells. The results for mammalian cells should carry more weight than those in bacterial cells, because mammalian cells are more relevant for understanding any potential effects in humans. Himmelstein *et al*. (2001a) tested the primary metabolite of chloroprene, (1-chloroethenyl)oxirane, and found it to be mutagenic in the absence of S9, suggesting that this metabolite may be the reactive agent in the Ames test; however, this epoxide metabolite was not genotoxic in mammalian cells *in vitro* (Chinese hamster V79 cells) (Himmelstein *et al*. 2001a). Therefore, the results from the Ames test may not be an accurate predictor of carcinogenicity of chloroprene, because glutathione and other detoxification pathways that would mitigate or eliminate the production of potentially active metabolites are not present in S9 microsome preparations at levels present in intact cells. Westphal *et al.* (1994) also found that addition of glutathione to the chloroprene/metabolite Ames tests significantly diminished the reported mutagenic activity. The absence of genotoxicity in intact mammalian cells systems and *in vivo* studies suggests that the bacterial mutagenicity data have limited relevance to the genotoxicity of chloroprene in humans. Critically, and as discussed below, *in vitro* systems do not have the normal levels of detoxifying

pathways found in intact mammalian cells to further metabolize/detoxify this primary metabolite.

4.2.4 Evidence does not support mutagenicity of chloroprene in vivo

The 2010 Review assumed that chloroprene is a point mutagen *in vivo* (in carcinogenicity bioassays with mutations identified in proto-oncogenes). Investigators study mutations in tumors at target sites to identify "mutagen fingerprints" for specific chemicals. As such, Sills *et al.* (1999, 2001) produced a proto-oncogene mutation profile for some target tumors in the mouse. A comparison of chloroprene and 1,3-butadiene indicated that the profile for chloroprene differed from that of 1,3-butadiene. In fact, the mutation rates in chloroprene-exposed animals were similar to mutation rates in control animals. Specific mutations were associated with chloroprene exposures across several different tumor types, but showed no dose-dependency. In contrast, the incidence of lung tumors increased with dose. This indicates that the lung tumors likely are independent of and unrelated to the mutations. These findings suggest that the underlying MOA is not the suspected K-*ras* mutation,[4] but rather a secondary MOA at target sites; for example, an MOA that follows a dose-dependent tumor response that is not associated with a corresponding dose-dependent increase in mutations, such as cytotoxicity-induced bronchiolar hyperplasia. If mutagenicity is the MOA, then mutation rates also should be dose-dependent. This is not the case for chloroprene, where mutations are not shown to be dose-dependent. Therefore, a different MOA is likely.

4.3 Evidence supports an alternative MOA for chloroprene based on cytotoxicity

Despite the inconsistencies in and questionable nature of the evidence for a mutagenic MOA, the 2010 Review never considered alternative MOAs for chloroprene. Considering alternative MOAs is recommended in US EPA's (2005) "Guidelines for Carcinogen Risk Assessment" and is consistent with recommendations by NRC (2011, 2014) for evidence integration and WOE analyses as specified in the Human Relevance Framework (Cohen *et al.* 2003, Meek *et al.* 2003, Cohen 2004, IPCS 2005, Boobis *et al.* 2006). US EPA (2005) guidelines noted that "where alternative approaches have significant biological support, and no scientific consensus favors a single approach, an assessment may present results using alternative approaches."

The likely alternative MOA for chloroprene is cytotoxicity, for which there are supportive experimental findings. At very high concentrations, chloroprene is toxic to animals, but does not demonstrate any genotoxicity (Shelby 1990), supporting an MOA based on target-site cytotoxicity. In mice, histopathology evaluations of chloroprene in target tissues are consistent with a non-genotoxic MOA. For example, the incidence of chloroprene-induced bronchiolar hyperplasia in the respiratory system follows the increased incidence of lung tumors, whereas the incidence of lung K-*ras* mutations (a precursor of many cancers) does not. Also, Melnick *et al.* (1996) reported that the toxicity and histopathology observed in

[4] Mutations of the k-*ras* gene are considered an essential step in the development of many cancers (*e.g.*, Jančík *et al.*, 2010).

chloroprene-treated F344 rats and B6C3F1 mice were substantially different from those seen in 1,3-butadiene exposed animals, suggesting an alternative MOA. In this case, a cytotoxicity-driven hyperplasia could be the cause, which can result from cell injury or death and subsequent tissue regeneration. Buzard *et al.* (1996) hypothesized that hyperplastic processes lead to selection of pre-existing oncogene and tumor suppressor gene mutations. Extrapolation from a target-site cytotoxic MOA involving cell proliferation and tumor promotion to other tumor sites is consistent with the attributes of chloroprene. It is important to note that the toxicity of chloroprene is observed at very high concentrations in mice and to a lesser extent in rats; however, it has been confirmed using a validated PBPK model that both species would be expected to be more sensitive to chloroprene exposure than humans. The differences in pharmacokinetics between mice, rats and humans helps to explain the lack of clear evidence of carcinogenicity in humans from epidemiology studies.

4.4 Conclusions

A critical evaluation of the cytotoxic and genotoxic profiles indicated that chloroprene acts through a MOA different from that of 1,3-butadiene, a known human carcinogen. Importantly, chloroprene's genotoxicity profile lacks several attributes necessary to conclude a mutagenic MOA:

- **Standard *in vivo* tests for genotoxicity are negative and unlike known carcinogens such as 1,3-butadiene**: Chloroprene, unlike 1,3-butadiene, is not genotoxic to somatic cells *in vivo.* The study results indicate that the epoxide metabolite of chloroprene is effectively detoxified under *in vivo* exposure conditions.
- **Consistent data are lacking for point mutation induction *in vitro* and *in vivo***: The evidence that chloroprene is able to produce point mutations *in vitro* (specifically in bacteria) is equivocal, and chloroprene did not induce mutations in cultured mammalian cells. There is a clear discordance between findings of *in vitro* point mutation, DNA adduct induction, and *in vivo ras* mutations in target site tumors, which indicate that the observation of these point mutations may not be relevant to the MOA for chloroprene-induced tumors.

Overall, unlike known carcinogens such as 1,3-butadiene, the evidence does not support a mutagenic MOA for chloroprene. Instead, the WOE supports an alternative MOA attributed to site-specific cytotoxicity. Thus, it is neither necessary nor appropriate to adjust the cancer unit risk based on a hypothesized mutagenic MOA, and deriving a new IUR based on an alternative MOA that can be scientifically substantiated is warranted.

5 EPIDEMIOLOGICAL EVIDENCE: OCCUPATIONAL STUDIES

5.1 Evaluation of the epidemiological studies

The 2010 Report classified chloroprene as "likely to be carcinogenic to humans" in part based on US EPA's interpretation of "an association between liver cancer risk and occupational exposure to chloroprene" and "suggestive evidence of an association between lung cancer risk and occupational exposure." As with the evaluation of the toxicological data, US EPA set forth criteria in the "Guidelines for Carcinogen Risk Assessment" (US EPA 2005) for the evaluation of epidemiological evidence, largely consistent with NRC recommendations (NRC 2014). While US EPA applied some of these criteria in the 2010 Review, US EPA did not present quality assessment and weighting of epidemiological evidence. Our application of these criteria led to largely opposite conclusions: appropriate weighing and synthesis of the epidemiological evidence demonstrated that chloroprene exposure is unlikely to cause lung or liver cancer at the occupational exposure levels encountered in the underlying studies. Furthermore, in contrast with US EPA's interpretation, the lack of any clear cancer risk is consistent with the results from the animal studies demonstrating significant differences across species in the carcinogenic potential of chloroprene, and the mechanistic evidence that humans are far less sensitive to chloroprene.

Using an approach consistent with US EPA (2005) and NRC (2014), Bukowski (2009) evaluated the quality of eight mortality studies of seven chloroprene-exposed cohorts from six countries (Table 5.1). Studies were assigned to categories of high, medium or low quality for each of ten quality criteria and a WOE assessment was performed. The four-cohort Marsh *et al.* (2007a, b) pooled study is the most methodologically rigorous epidemiology study conducted to date. This study has the largest overall cohort size and the most rigorous follow-up. Based on the large cohort size, the Marsh study has the highest statistical power (see Table 5.2). Finally, the Marsh study has the most comprehensive exposure assessment, including assessment of exposure to potentially confounding agents such as vinyl chloride.

Table 5.1. Quality Rankings for Cohort Studies of Cancer Risks from Occupational Chloroprene Exposure

US EPA Criteria	Marsh et al. (2007 a,b) Study				Other Studies			
	Kentucky [1]	North Ireland [1]	Louisiana [1]	France - Mort* [1]	Armenia [2]	France - Incid** [3]	Russia [4]	China [5]
Clear objectives	H‡	H	H	H	H	H-M	H	M
Comparison groups	H	H-M	H-M	M	M	M	M-L	L
Exposure	H	H	H	H	M	M	L	L
Follow-up	H	H-M	H	H-M	M-L	M-L	M-L	M-L
Case ascertainment	H	H-M	H-M	H-M	M	M	M	H-M
Control of bias	H-M	H-M	H-M	M	M-L	M	M	M-L
Sample size	H	H	M	L	M-L	L	H-M	M-L
Data collection and evaluation	H	H	H	H	M	M	M-L	M-L
Adequate response	H	H	H	H	M	M	M	H-M
Documentation of results	H	H	H	H	M-L	M	M	L
Overall rank (1=best)	**1**	**2**	**3**	**4**	**5**	**5**	**5**	**6**

Source: Bukowski 2009 * Mort=Mortality ** Incid=Incidence ‡ Subjective estimate of study quality for each specific criterion H=high, M=medium, L=low; 1 – Marsh *et al*. 2007; 2 – Bulbulyan *et al.* 1999; 3 – Colonna and Laydevant 2001; 4 – Bulbulyan *et al*. 1998; 5 – Li *et al*. 1989

Table 5.2. Relative Size of Marsh *et al*. (2007a, b) Study Compared with Other Available Studies

Study	Subjects (Person-years)	Lung Cancer Deaths	Liver Cancer Deaths
Bulbulyan et al. 1998	5185 (70,328)	31	10
Bulbulyan et al. 1999	2314 (21,107)	3	3
Colonna and Laydevant 2001	717 (17,057)	9	1
Lee and Selevan 1982	Should not be included in the 2010 Review		
Li et al. 1989	1258 (20,105)[a.]	2	6
Total Other Studies	**9474 (128,597)**	**45**	**20**
Marsh et al. 2007a (L)	5507 (197,010)	266	17
Marsh et al. 2007a (M)	4849 (127,036)	48	1
Marsh et al. 2007a (P)	1357 (30,660)	12	0
Marsh et al. 2007a (G)	717 (17,057)	10	1
Total Marsh et al. (2007a, b)	**12,430 (372,672)**	**336**	**19**
Combined Studies	**21,904 (501,269)**	**381**	**39**
Marsh et al. (2007a, b) / Combined Studies	**57% (74%)**	**88%**	**49%**

Previously, Rice and Boffetta (2001) reviewed the published epidemiological studies of chloroprene exposed cohorts. Their review included cohorts in the US (Pell 1978), China (Li *et al*. 1989), Russia (Bulbulyan *et al*. 1998), and Armenia

(Bulbulyan *et al*. 1999) and noted significant methodological limitations in these studies, including unclear documentation for cohort enumeration, inadequate reference rates for standardized ratios, a lack of detailed histopathology of liver cancer cases, and limited or no information on potential co-exposures. They also remarked that the occupational chloroprene exposure assessment was poor for all published studies, and the statistical power of the available studies was low due to the small number of observed cancers of interest. Notably, one of the co-authors of the critical review (Boffetta) was also a contributing author of the cohort studies in Russia and Armenia (Bulbulyan *et al*. 1998 and Bulbulyan *et al.* 1999, respectively).

To date, the identified limitations of the studies of Chinese, Russian, and Armenian cohorts remain unaddressed, and most have not been updated. Only the original studies of the US cohort from Louisville, Kentucky (Pell 1978, Leet and Selevan 1982) have been updated and improved. Substantial improvements included detailed descriptions of the cohorts, appropriate comparisons to local cancer rates, an improved exposure assessment both for chloroprene and associated co-exposures (such as vinyl chloride), appropriate follow-up times to capture all potential cancers, appropriate and valid determination of cancer cases, and well-documented methods and results (Marsh *et al.* 2007a, b). A comparison of the study limitations for key quality criteria across the different cohorts is summarized in Table 5.3, and discussed in detail in the next section.

Table 5.3. Comparison of Key Study Criteria across Epidemiological Studies

Key Criteria	US and Europe (Marsh et al. 2007a,b)	Armenia (Bulbulyan et al. 1999)	Russia (Bulbulyan et al. 1998)	China (Li et al. 1989)
Sample Size	French, Irish and US 12,430 (Kentucky ~200,000 person-years)	2,314	5,185	1,258
Follow-up	1949–2000	1979–1993	1979–1993	1969–1983
Exposure Assessment	Exposure modeling – 7 categories	Index (none, low, high)- before/after 1980	Index (none, med, high)- IH (inadequate) + job	High vs. low based on recall
Baseline rates	National, local plant area counties	Armenian rates	Moscow rates	From "local area" 1973–1975
	1960–1994	1980-1989	1979–1993 or	expected lung cancers: 0.4
			1992–1993 (liver)	
Confounding	Used local rate comparisons;	Alcohol use (high cirrhosis rates) and smoking prevalent	Alcohol use (high cirrhosis rates) and smoking;	Hepatitis B and aflatoxin;
	Low prevalence of other liver cancer risk factors		Co-exposure to VCM	Co-exposures to VCM

IH: Industrial hygiene
VCM: vinyl chloride monomer

5.2 Important limitations of the epidemiology literature

The 2010 Review considered lung and liver cancer mortality reported in studies of occupational cohorts from several countries published over 30 years: Pell (1978), Leet and Selevan (1982), Li *et al*. (1989), Bulbulyan *et al*. (1998, 1999), Colonna and Laydevant (2001), and Marsh *et al*. (2007a,b).

Cohort studies comprise a set of data distributed over time to address a hypothesized exposure-disease association (Checkoway *et al*. 2004). In synthesizing results of several cohort studies – or when conducting meta-analyses of such results – it is important to verify that each study cohort is an independent sample and that analytic results are independent, *i.e*., there should be no overlap (e.g., Greenland and O'Rourke 2008). Especially for outcomes with long latency periods and high case-fatality, such as lung and liver cancers, only the most recent and most complete (and non-overlapping) results from cohorts with multiple follow-up periods should be used. Updated results always have more observed person-years at risk and almost always include larger numbers of the health outcome of interest, increasing statistical stability and reducing the probability of chance findings.

The epidemiological literature on chloroprene consists of seven published reports based on nine distinct cohorts. In the 2010 Review, however, *each published epidemiological study* was included as if it were independent, including early results from overlapping or updated cohorts. Specifically, the early results from the Pell (1978) and Leet and Selevan (1982) were included in the most recent update (Marsh *et al*. 2007a, b). Therefore, the Pell (1978) and Leet and Selevan (1982) studies should not have been considered as independent evidence, since all of their cancer deaths were included in the Marsh (2007 a, b) update.

Additionally, the Chinese, Russian, and Armenian studies have serious limitations, as documented by several authors including Rice and Boffetta (2001), Acquavella and Leonard (2001), and Bukowski (2009). As noted above, these studies have not been updated and the noted limitations remain unaddressed. These studies therefore should be given less weight in the synthesis of evidence.

The study of Chinese workers (Li *et al*. 1989) suffered from small numbers of workers, inadequate reference population mortality rates for statistical comparisons, and a lack of adjustment for known causes of lung and liver cancers. The researchers ascertained mortality among 1,213 workers for a 14-year period from 1969 through 1983 and reported 6 deaths due to liver cancer and 2 deaths due to lung cancer. However, they used local mortality rates for only a three-year period (1973 to 1975) to estimate expected numbers of specific cancers. For rare events such as any specific cancer, estimates based on small numbers will be inherently imprecise. Li *et al*. (1989) reported 2.5 and 0.4 expected liver and lung cancer deaths, respectively, among all cohort members followed between 1969 and 1983. The limited number of observed liver and lung cancer deaths divided by the very small expected numbers produced highly imprecise standardized mortality ratios (SMRs) with very large confidence limits. Furthermore, estimates for liver and lung cancer incidence are higher among Chinese men (in 2002, liver cancer mortality was 38 per 100,000 persons per year, and lung cancer mortality was 42 per 100,000 persons per year) and women (liver cancer, 14 per 100,000 persons

per year, and lung cancer, 19 per 100,000 persons per year) (Parkin *et al*. 2005) compared to the rest of the world. In the most high-risk areas of China, 1 in 10 people died of liver cancer (Hsing *et al*. 1991). The major causes of liver cancer in China are chronic infection with hepatitis B virus and aflatoxin B1, in addition to the rising prevalence of alcohol consumption and tobacco smoking (Chen *et al*. 2003, Stuver and Trichopoulos 2008, Lee *et al*. 2009). In contrast, in the US in the years 2009–2013, there were an estimated 9 liver cancer deaths per 100,000 men and 4 liver cancer deaths per 100,000 women per year (SEER 2017). Therefore, observational studies of liver cancer mortality within this Chinese population should control for known causes of these cancers as potential confounding factors. However, the authors of the Chinese study did not control for these confounding factors, and US EPA did not consider the lack of control for confounders when evaluating the quality and weight of the evidence from this study.

Similar to the Li *et al*. (1989) study, Bulbulyan and colleagues (1998) calculated expected numbers of liver cancers using mortality and incidence rates for Moscow for only two years (1992 to 1993), resulting in imprecise reference rates and unstable results. Cancer mortality data from 36 European countries, including the Russian Federation, showed that liver cancer mortality rates among women increased from 1960, peaked during the late 1970s, and declined to their lowest levels during the early 1990s, the period chosen for the study's reference mortality rates (Levi *et al*. 2004). In addition, the Armenian cancer registry is incomplete and may have misclassified the histopathology of reported liver cancers for the general population. Using a reference population with incomplete numbers and mortality rates representative of only a small time period would underestimate the expected incidence and mortality of liver cancer, resulting in over-estimates of the risk estimates. In light of the small numbers and the likelihood that chance may be an explanation for these estimates, the imprecise numbers reported in Bulbulyan *et al*. (1999) and repeated in Zaridze *et al*. (2001) should be viewed skeptically and given little, if any, weight.

The Russian and Armenian cohorts also suffered from inadequate consideration of other major causes of liver cancer. In the populations represented in these cohorts, there is a high incidence of alcoholic cirrhosis, a well-known precursor for liver cancer (London and McGlynn 2006). There were 11 deaths from cirrhosis of the liver (3 in males and 8 in females) recorded for the Russian cohort. In the Armenian cohort, 32 cases of cirrhosis of the liver were reported (27 in males and 5 in females). Alcohol consumption and smoking are well known risks factors for liver cancer, and these factors were not adjusted for in the eastern European cohort studies (Keller 1977, Makimoto and Higuchi 1999, Lee *et al*. 2009). A report by the World Health Organization (WHO 2009) reported a prevalence of 70% and 27% for current tobacco use among Russian men and women, respectively, and noted high levels of alcohol consumption for the general population. The prevalence of current tobacco use among Armenian men is also very high at 55% (WHO 2009). Proper control for these causes was not possible, increasing the likelihood of confounding and thus rendering the results unreliable.

Previous reviews have critiqued the Chinese, Russian, and Armenian studies for inadequate descriptions of the source population rates used to calculate SMRs and standardized incidence ratios (SIRs) (Rice and Boffetta 2001). Another important

methodological concern for the interpretation of SMR and SIR estimates is that when they are based on very small expected values (*i.e.*, less than two), they indicate small population size and/or short follow-up, contributing to unstable estimates (Checkoway, 2004). As such, findings from these studies are not reliable and should carry little if any weight in evaluating cancer causation.

Taken together, the epidemiological studies evaluated in the 2010 Review do not establish a clear causal connection between occupational chloroprene exposure and liver and lung cancers. Consequently, the US EPA's interpretation of the epidemiological evidence as justifying a classification of chloroprene as "likely to be carcinogenic to humans" is questionable. In particular, US EPA's giving the same weight to the large and more robust Marsh *et al*. (2007a, b) epidemiological studies as it gave to the lower quality, lower power studies is inappropriate. Although the Marsh *et al*. (2007a, b) studies have limitations typical of all historical cohort studies, they are the largest studies of potential cancer outcomes with the most complete documentation of exposure. These studies also were designed and conducted specifically to address the limitations previously noted, making the evidence from the Marsh *et al*. (2007a, b) studies far more valid and informative than that from the other studies evaluated by US EPA. The review by Bukowski (2009) (represented in Table 5.1) ranked the study by Marsh *et al.* (2007a, b) as having the highest relative strength based on the same criteria for evaluation listed in the US EPA's "Guidelines for Carcinogen Risk Assessment" (US EPA 2005) and consistent with NRC recommendations (NRC 2011, 2014), and it therefore should be given the greatest weight.

5.3 The Marsh *et al*. (2007a, b) studies do not show a causal link between occupational exposure to chloroprene and increased cancer risks

The Marsh *et al*. (2007 a, b) studies, the most robust epidemiological studies of occupational chloroprene exposure, found no excess of lung or liver cancers (Marsh *et al*. 2007a, b). The 2010 Review, however, stated, "The study involving four plants (including the Louisville Works plant included in the Leet and Selevan (1982) study by Marsh *et al*. (2007a, 2007b), which had the largest sample size and most extensive exposure assessment, also observed increased relative risk estimates for liver cancer in relation to cumulative exposure in the plant with the highest exposure levels (trend p value = 0.09, relative risks [RRs] 1.0, 1.90, 5.10, and 3.33 across quartiles of exposure)." However, the interpretation of these relative risks is more complex than US EPA stated, as the rate of liver cancer deaths among workers was not different from that in the general population.

As shown in Table 5.4, Marsh *et al*. (2007a) computed standardized mortality ratios (SMRs) using national and regional standard populations for the overall cohorts, for selected demographics (males, females, blue-collar workers), and for work histories and exposure factors. The authors concluded that occupational exposures to chloroprene at the levels encountered by each of the cohorts did not show evidence of elevated risk of cancer, including liver cancer.

In a separate publication, Marsh *et al.* (2007b) reported exposure-response data for chloroprene exposure and cancer. In Table 5.5 and Figure 5.1, results for the Louisville plant are shown, including both the internal analyses (relative risks or RRs) and external analyses (SMRs) which are based on comparisons with county

populations. The RRs are the values that US EPA focuses on in their assessment of potential liver cancer risks. However, as noted by Marsh *et al.*, "The elevated RRs result mainly from the exceedingly low death rates associated with the baseline categories of each measure, as reflected by the correspondingly low SMRs (*i.e.*, the RR for a given non-baseline category is roughly related to the ratio of the corresponding SMR for that category to the SMR for the baseline category)."

Table 5.4. Reported Observed Liver Cancer Cases, Expected Counts, and Standardized Mortality Estimates for the Marsh *et al.* 2007a Study

Study Cohort	Observed	Expected*	SMR or SIR	95% Confidence Limits		p-value
				Lower	Upper	
Louisville	17	16.35	1.04	0.61		
Maydown	1	4.17	0.24	0.01		
Pontchartrain	0	--	--	--	--	--
Grenoble	1	1.79	0.56	0.01		
Louisville Subcohorts (local reference)						
Full Cohort	17	18.89	0.9	0.53	1.44	0.78
White race	16	15.69	1.02	0.58	1.65	0.99
Non-White race	1	3.13	0.32	0.01	1.77	0.36
Males	16	17.98	0.89	0.51	1.45	0.75
Females	1	0.94	1.06	0.03	5.93	0.99
Blue collar	17	18.28	0.93	0.54	1.49	0.89
Short-term worker	4	8.16	0.49	0.13	1.26	0.18
Long-term worker	13	10.74	1.21	0.64	2.07	0.57
Duration of employment						
< 5years	4	8.16	0.49	0.13	1.25	0.18
5-19 years	6	3.57	1.68	0.62	3.66	0.30
20+ years	7	7.14	0.98	0.4	2.03	0.99
Time since 1st employment						
< 20 years	1	1.79	0.56	0.01	3.11	0.93
20-29 years	3	3.3	0.91	0.19	2.66	0.99
30 + years	13	13.68	0.95	0.5	1.62	0.99
CD exposure status						
Exposed	17	18.89	0.9	0.53	1.44	0.78

From Marsh *et al.* 2007a

Table 5.5. Exposure-Response Analysis for Chloroprene and Liver Cancers, Based on Internal (Relative Risks) and External (Standardized Mortality Ratio) Estimates, Louisville Plant

Liver cancer	Deaths	Internal Analysis			External Analysis	
		# cases	RR (95% CI)	p-value	Person-years	SMR (95% CI)
Exposure Duration (years)						
<10	6	1500	1.00	Global=0.24	131276	0.61 (0.22-1.32)
10-19	4	216	3.85 (0.75-17.09)	Trend=0.36	30404	2.08 (0.57-5.33)
20+	7	965	1.75 (0.49-6.44)		36239	0.99 (0.40-2.04)
Average Intensity of Exposure (ppm)						
<3.62	3	714	1.00	Global=0.22	69274	0.62 (0.13-1.80)
3.62 - 8.12	7	568	3.81 (0.77-25.76)	Trend=0.84	27933	1.73 (0.70-3.56)
8.12-15.99	3	388	1.84 (0.22-15.74)		28689	0.94 (0.19-2.74)
16.0+	4	1011	1.31 (0.20-10.07)		72023	0.59 (0.16-1.52)
Cumulative exposure (ppm-years)						
<4.75	2	744	1.00	Global=0.17	68918	0.43 (0.05-1.55)
4.75-55.19	3	725	1.9 (0.21-23.81)	Trend=0.09	56737	0.59 (0.12-1.74)
55.91-164.0	7	653	5.1 (0.88-54.64)		39840	1.62 (0.65-3.33)
164.0+	5	559	3.33 (0.48-39.26)		32424	1.00 (0.33-2.34)

From Marsh et al. 2007b; Table 4
CI: confidence interval
ppm: parts per million

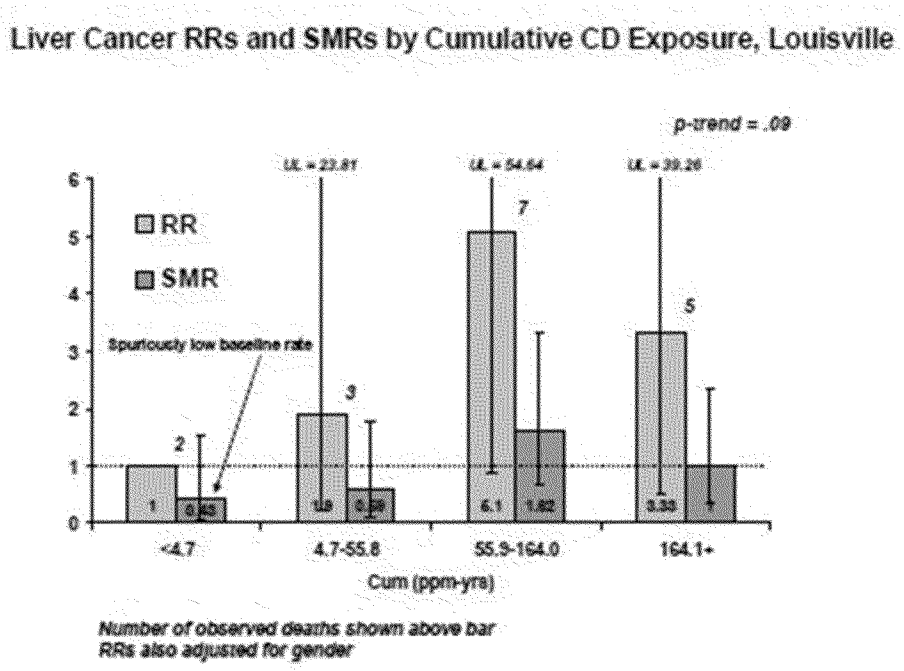


Figure 5.1 Liver Cancer RRs and SMRs by Cumulative Chloroprene Exposure, Louisville

US EPA noted that 3 of the 15 subgroups in Table 5.5 had SMRs greater than 1.00, and inferred from these a likely causal relationship between chloroprene exposure and cancer. However, none of these three SMRs reached statistical significance *(i.e.*, the findings may have been due to chance). In fact, the 95% confidence intervals in Table 5.5 show up to a 10-fold margin of error around the estimated SMRs, underscoring the statistical instability and uncertainty of the risk estimates for these subgroups. In addition, as noted by Marsh *et al*. (2007b), the risk estimates were derived comparing risk from higher exposure groups to risk in the group with the lowest exposure, which had only two liver cancer deaths. The occurrence of only two liver cancer deaths in the lowest exposure group represented a clear deficit in the expected rate of liver cancer, as demonstrated by the SMR (Table 5.5). Comparison to a group with a deficit (most likely due to chance given the small numbers) led to the spurious appearance of an increased risk among the more highly exposed groups. Overall, the chloroprene exposed workers had only about 90% of the expected mortality rate (17 observed with about 19 expected), based on a non-exposed population reference rate (Table 5.4).

Taken as a whole, the epidemiological evidence on chloroprene and cancer is insufficient to conclude that chloroprene is a human carcinogen. The study by Marsh *et al*. (2007a, b) is the largest and methodologically the strongest and, therefore, should carry the greatest weight in integrating the epidemiological evidence for chloroprene. This epidemiological evidence is consistent with the toxicological hypothesis that humans are less sensitive than animals to the possible carcinogenic effects of chloroprene, and also supports the conclusion by Allen *et al*. (2014) that a modified cancer unit risk that accounts for animal-to-human extrapolations is needed.

6 CANCER CLASSIFICATION FOR CHLOROPRENE

The 2010 Review determined that chloroprene was "likely to be carcinogenic to humans" based on EPA's conclusions of (1) statistically significant and dose-related information from the NTP (1998) chronic inhalation bioassay data demonstrating the early appearance of tumors, development of malignant tumors, and the occurrence of multiple tumors within and across animal species; (2) evidence of an association between liver cancer risk and occupational exposure to chloroprene; (3) suggestive evidence of an association between lung cancer risk and occupational exposure; (4) a proposed mutagenic mode of action (MOA); and (5) structural similarities between chloroprene and known human carcinogens, 1,3-butadiene and vinyl chloride. As has been demonstrated in this report, three of the five EPA conclusions are not supported by the weight of evidence, and the fourth—structural similarities —has been shown not to be informative, as the chemicals demonstrate different modes of action. Based on the limited evidence remaining to support the potential carcinogenicity of chloroprene, we conclude that a more appropriate classification of chloroprene is "suggestive evidence of carcinogenic potential."

To classify a chemical as "likely to be carcinogenic to humans," US EPA notes that "this descriptor is appropriate when the weight of the evidence is adequate to demonstrate carcinogenic potential to humans but does not reach the weight of evidence for the descriptor "carcinogenic to humans (US EPA, 2005)." Adequate evidence consistent with this descriptor covers a broad spectrum and as noted by US EPA (2005), "choosing a descriptor is a matter of judgment and cannot be reduced to a formula. Each descriptor may be applicable to a wide variety of potential data sets and weights of evidence." Strong evidence for carcinogenicity in humans is not needed; however, the weight of evidence is still required to support the classification descriptor.

In the 2010 Review, the weight of evidence narrative provided for chloroprene to support the descriptor of "likely to be carcinogenic to humans" was limited to a check-list provided above (US EPA, 2010a, pg. 96 and Table 4-39). However, in reviewing the underlying data for the evidence presented in this checklist, we note that only two of the five can be substantiated: (1) statistically significant and dose-related information from the NTP (1998) chronic inhalation bioassay data, and (5) structural similarities between chloroprene and known human carcinogens, 1,3-butadiene and vinyl chloride.

We have demonstrated considerable misinterpretation in the 2010 Review of the available science to support other items on the checklist. For example, the epidemiological evidence, based on an appropriate weight of evidence approach, fails to demonstrate clearly increased risks among exposed occupational groups and the general population, and a weak difference between exposed and unexposed workers reflecting a deficit among the least exposed (see Section 5). The claim that chloroprene is mutagenic is not supported by the overall evidence from the available data, as discussed in Section 4. Although there are structural similarities of chloroprene and 1,3-butadiene and vinyl chloride, the toxicological evidence including possible modes of action (MOAs) demonstrate substantial differences between chloroprene, vinyl chloride, and 1,3-butadiene.

Most importantly, the narrative does not include discussion of critical uncertainties in relying on the mouse data from NTP (1998) to predict the potential for carcinogenic risk in the humans, given ample evidence of important pharmacokinetic differences between mice and other species. In fact, the NTP study and other animal studies show that there is little evidence of consistent tumorgenicity across species other than the mouse and in particular the hamster (see Section 3). This difference can clearly be explained by evidence of differences in the pharmacokinetics of chloroprene across species. In addition, consideration of the lack of evidence of the carcinogenicity of chloroprene from human studies and the risks that would be predicted relying on the results from human studies (see Section 11) further indicate that a classification of "likely" carcinogen is inappropriate.

The weight of evidence supports a reclassification. According to US EPA (2015) the updated classification narrative should address the following:

- The weight of the evidence should be presented as a narrative laying out the complexity of information that is essential to understanding the hazard and its dependence on the quality, quantity, and type(s) of data available, as well as the circumstances of exposure or the traits of an exposed population that may be required for expression of cancer.
- In borderline cases, the narrative explains the case for choosing one descriptor and discusses the arguments for considering but not choosing another.
- The descriptors can be used as an introduction to the weight of evidence narrative. The complete weight of evidence narrative, rather than the descriptor alone, provides the conclusions and the basis for them.

A complete and accurate narrative also should capture and interpret all documented major uncertainties in the evidence as it relates to the classification of chloroprene. Transparent documentation of methods, data and assumptions, coupled with an accurate and informative classification of the weight of evidence is needed. Considering the misinterpretation of some data and the uncertainty in relying on responses in the mouse to be predictive of the potential for carcinogenicity in humans, the current classification of "likely to be carcinogenic to humans" unduly raises public health concerns. We conclude that a descriptor of "suggestive to be carcinogenic to humans" is more representative of the weight of evidence and uncertainties associated with relying significantly on results from a species for which there is evidence of differences that explain the observed sensitivity compared to the human.

7 US EPA DERIVATION OF THE CHLOROPRENE IUR

As described in Section 3, US EPA relied primarily on the findings of a two-year inhalation study conducted by the NTP (1998) in B6C3F1 mice and F344/N rats. Trochimowicz *et al.* (1998) also conducted studies in Wistar rats and Syrian hamsters. The results of the NTP (1998) and Trochimowicz *et al.* (1998) studies showed that the mouse is the most sensitive species to chloroprene among the species tested. US EPA selected the results from the female mouse to be the basis for deriving the chloroprene IUR. However, given the differences in response in the mouse compared to other laboratory species, US EPA should have considered the potential for differences in pharmacokinetics to better characterize and explain the cross-species differences. Although this source of bias is likely the largest and most significant, US EPA applied a number of additional assumptions in deriving the chloroprene IUR that lead to conservative bias and unsupported uncertainty in the IUR. The following sections highlight these key sources of uncertainty.

7.1 US EPA's dose-response modeling applied overly conservative methodology

US EPA determined the point of departure (POD)[5] using dose-response modeling to derive the IUR. Specifically, US EPA estimated the effective dose at a specified level of response (a benchmark dose concentration associated with a 10% risk level [BMD_{10}]) and its lower-bound based on the lower 95% confidence interval of the BMD_{10} ($BMDL_{10}$) for each chloroprene-induced tumor type in the mouse. Having determined that chloroprene was more potent in inducing tumors in mice than in rats, US EPA did not consider the rat data further in developing the IUR. US EPA further noted that the observed differences may be due to species differences in metabolism.

US EPA modeled each mouse tumor endpoint reported in NTP (1998) separately using the US EPA multistage Weibull time-to-tumor model. The multistage Weibull model has the following form:

$$P(d,t) = 1 - \exp[-(b_0 + b_1 d + b_2 d^2 + \ldots + b_k d^k) \times (t - t_0)^c]$$

where P(d,t) represents the lifetime risk (probability) of cancer at dose d (the human equivalent exposure in this case) at time t (a human lifetime in this case); parameters $b_i \geq 0$, for I = 0, 1, ..., k; t is the time at which the animal's tumor status, either no tumor, tumor, or unknown (missing or autolyzed) was observed; t_0 is the latency of response; and c is a parameter which characterizes the change in response with age. For the analysis performed in the 2010 Review, the latency (t_0) was set to zero for all models. The power term parameter c is normally a parameter that is estimated by the BMD software. For some tumors, the model software was unable to calculate this parameter and US EPA had to estimate this value (*e.g.*, for forestomach tumors).

In the modeling, US EPA conservatively considered all tumor types, both benign and malignant. US EPA also assumed that the dose-response was linear in the low

[5] A POD is defined as the point on a dose-response curve that marks the beginning of a low-dose extrapolation. This point is typically a lower bound, expressed in human-equivalent terms, near the lower end of the observed range. This POD is used to extrapolate to lower exposures to the extent necessary.

dose range, based on the assumption that chloroprene has a mutagenic MOA. This approach is not justified by the available scientific evidence; therefore, the assumption of linearity inappropriately adds another level of uncertainty to the IUR.

7.2 Extrapolation from animals to humans should have included use of a PBPK model

In the 2010 Review, US EPA did not use a PBPK model for chloroprene to adjust for differences across species, even though a model was available. At the time, US EPA stated that it did not have sufficient data to validate the model. However, all of the quantitative data necessary to refine and verify the critical metabolic parameters for the existing peer-reviewed model for chloroprene (*i.e.*, Himmelstein *et al*. 2004b) were available and could have been applied to adjust the IUR. Further, since the release of the 2010 Review, additional peer-reviewed studies have been published, demonstrating consistent results and validating the use of the model for dose-response modeling and determination of an appropriate human equivalent concentration for the human IUR (Yang *et al*. 2012, Thomas *et al*. 2013, Allen *et al*. 2014).

Instead of using a PBPK model to account for differences between humans and animals, US EPA used a default approach that entails applying a dosimetry adjustment factor (DAF) that accounts for some differences in the blood:air partitioning in animals compared to humans. US EPA used a DAF of 1.0 (essentially assuming equivalence), based on the unsubstantiated assumption that all the lung tumors observed were the result of systemic effects from chloroprene exposures. US EPA provided no evidence to support the assumption that tumors in the lungs of mice are the result of systemic effects, rather than the more plausible portal-of-entry effects that would result from direct contact of chloroprene with lung tissue.[6] As noted by US EPA (2010a), "treating lung tumors as systemic effects returns the highest composite unit risk (approximately 60% greater than if lung tumors are treated as portal-of-entry effects)."

7.3 Deriving a composite IUR based on multiple tumors is not scientifically supported

Another source of overly-conservative bias in the derivation of the IUR is the use of a composite value of multiple tumor types instead of the standard approach of using the most sensitive species, gender, and endpoint(s). The use of the composite value for chloroprene is not valid. While US EPA assumed statistical independence of different tumor types based on a hypothesized MOA for chloroprene involving the production of epoxide metabolites, the underlying data do not demonstrate mechanistic or biological independence. The mechanism of action in multiple tissues could also be due to dependent events; for example, a liver tumor could be dependent on the generation of the same metabolite as that needed for the development of a lung tumor. Figure 7.1 illustrates how US EPA's assumption of adding risk across multiple tumor sites overestimates the potential overall cancer risk. Figure 7.1 also shows the considerable non-random distribution

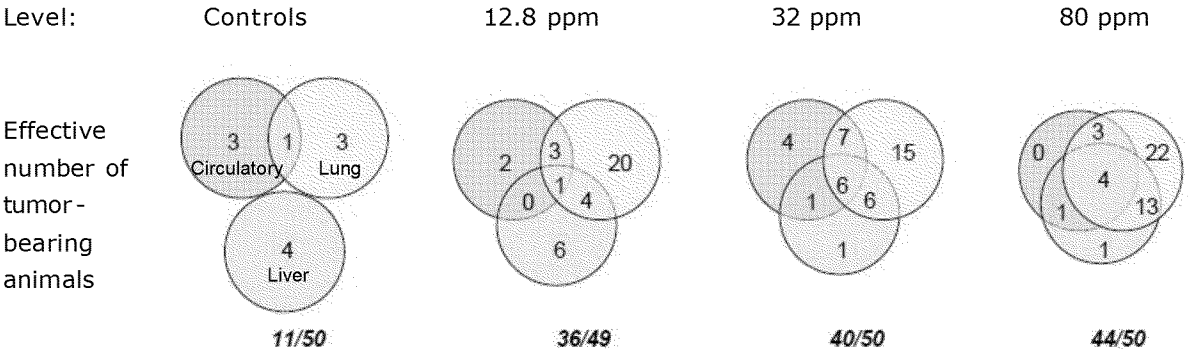
[6] A portal-of-entry effect is a localized effect that occurs at the point at which a substance enters the body (*e.g.*, via inhalation there would be effects on the respiratory system). Systemic effects, on the other hand, are effects that occur in other organs of the body distant from the portal-of-entry (*e.g.*, effects on the liver following inhalation of the substance).

of tumors in the animals bearing multiple tumors. Therefore, when US EPA assumed independence based on an unknown MOA, this inflated the effective number of animals developing tumors and overstated the carcinogenicity of chloroprene. US EPA recognized that the assumption of independence could not be verified, and that if this assumption did not hold, it indeed would overestimate risk (US EPA 2010a), in this case by another 50%.

In calculating the composite estimated IUR, US EPA also assumed that the IURs were normally distributed around the mean with a 95% upper confidence limit that represents the composite estimate. However, there is no evidence to support a normality assumption either in the benchmark dose (BMD) or the IUR, which adds to the uncertainty in the risk estimate.

Based on the US EPA approach of summing IURs for individual tumor types, the estimated composite inhalation IUR for female mice (which were more sensitive to chloroprene than male mice) was increased by approximately 50%, from 1.8×10^{-4} for the most sensitive endpoint (lung tumors in female mice) to 2.7×10^{-4} per µg/m³ for all tumors combined. US EPA rounded this to a single significant figure, resulting in an even more conservative IUR for continuous lifetime exposures to adult humans of 3×10^{-4} per µg/m³.

NTP Data



US EPA Approach

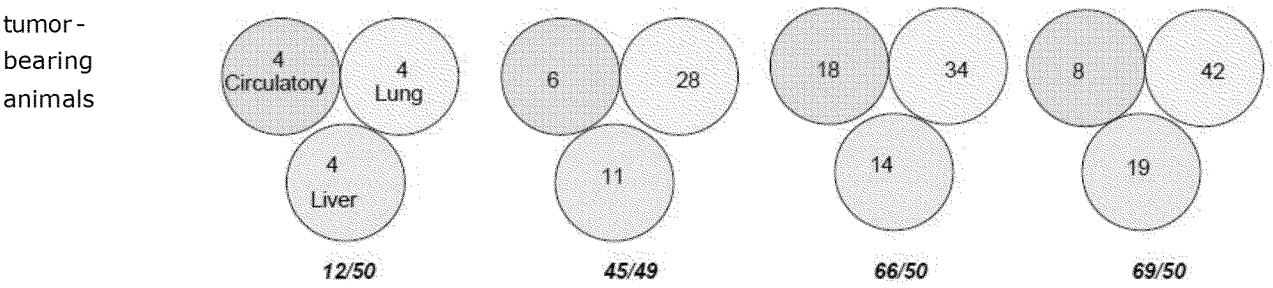


Figure 7.1. Illustration of How US EPA's Approach of Summing Individual Tumor Potencies Overestimates Total Tumor Potency in Female Mice by Assuming Independence.

7.4 IUR adjustment for early life susceptibility is not appropriate

In the final step, US EPA applied an age-dependent adjustment factor (ADAF) to account for early-life susceptibility, because of a hypothesized mutagenic MOA. This yielded a final adjusted unit cancer risk of 5×10^{-4} per µg/m^3. This adjustment reflects the use of several sensitivity adjustments for different life-stages, which are applied for presumed mutagenic compounds as specified in US EPA's "Supplemental Guidance for Assessing Susceptibility From Early-Life Exposure to Carcinogens" (US EPA 2005). Specifically, as described in the US EPA (2005 b) guidance, US EPA applied the default ADAFs and their age groupings of 10 for <2 years, 3 for 2 to <16 years, and 1 for 16 years and above. The calculations are shown below.

Risk for birth through <2 yr $= 3 \times 10^{-4}$ per µg/m^3 $\times$ 10 $\times$ 2 yr/70 yr $= 8.6 \times 10^{-5}$ per µg/m^3

Risk for ages 2 through <16 $= 3 \times 10^{-4}$ per µg/m^3 $\times$ 3 $\times$ 14 yr/70 yr $= 1.8 \times 10^{-4}$ per µg/m^3

Risk for ages 16 until 70 $= 3 \times 10^{-4}$ per µg/m^3 $\times$ 1 $\times$ 54 yr/70 yr $= 2.3 \times 10^{-4}$ per µg/m^3

The individual risk estimates were then summed to obtain the final lifetime (70 years) IUR for chloroprene:

$$\text{Risk} = 8.6 \times 10^{-5} + 1.8 \times 10^{-4} + 2.3 \times 10^{-4} = 5.0 \times 10^{-4} \text{ per } \mu g/m^3$$

As with the calculation of a composite IUR (which was increased by 67% based on the combination of tumors), US EPA's assumption of a mutagenic MOA increased the calculated IUR by another 67%. Taken together, these assumption s increased the IUR calculation to 178% of the IUR calculated based on the most sensitive species at the most sensitive site. As discussed in detail in Section 4, the ADAF adjustment is not applicable to chloroprene because there is insufficient evidence of a mutagenic MOA for chloroprene.

7.5 Summary of US EPA's derivation of the chloroprene IUR

The chloroprene IUR derived in the 2010 Review was based on the following assumptions, some of which are not scientifically substantiated:

1. US EPA selected the most sensitive species, female B6C3F1 mice, based on the results from the NTP (1998) study;
2. US EPA assumed lung tumors in mice to be a systemic lesion and not a portal-of-entry effect, resulting in a minimal dosimetric adjustment for extrapolating from animals to humans (i.e., application of a DAF =1);
3. US EPA calculated a composite risk estimate based on multiple tumor sites, although multi-tumor data were inconsistent and relatively weak for most tumor sites;
4. US EPA rounded the IUR prior to applying the ADAF, increasing the IUR further; and
5. US EPA applied an ADAF based on the assumption of a mutagenic MOA.

Table 7.1. Conservative Assumptions in the Calculation of the Chloroprene IUR

Step	IUR per µg/m³	Basis	Amount of overestimate	Cumulative overestimate
Most sensitive endpoint/species (portal -of-entry DAF=1.7)	1.06×10^{-4}	Lung tumors in female mice as a portal-of-entry effect		
Most sensitive endpoint/species (systemic lesion DAF=1)	1.8×10^{-4}	Lung tumors in female mice as a systemic effect	1.7	
Multiple tumor adjustment	2.7×10^{-4}	Multiple tumors	1.5	
Rounding	3×10^{-4}	Rounding	1.1	2.8
Application of ADAF	4.5×10^{-4}	Adjustment (without rounding)	1.5	4.2
Application of ADAF	**5×10^{-4}**	**Adjustment (with rounding)**	**1.7**	**4.8**

Combined, these assumptions contribute to a risk estimate that is over-estimated by about a factor of 5 (Table 7.1). However, these assumptions contribute only to a small overestimate compared to consideration of the documented differences across species, which was reported by Allen *et al.* (2014) and confirmed by our own calculations of an updated IUR. Consideration of pharmacokinetic differences across species indicate that the chloroprene IUR is likely overestimated by two orders of magnitude.

7.6 Replication of US EPA's dose-response modeling

The 2010 Review used the results from the NTP (1998) study in mice to calculate multiple PODs for derivation of the composite IUR (see previous section). US EPA focused specifically on the female mouse as this was the most sensitive species and gender, but assumed that this animal model was directly applicable to humans. Further, US EPA assumed a default linear dose-response and applied the multistage Weibull model, which accounts for the influence of competing risks (such as early death) and for the occurrence of multiple tumors, some of which are incidental (benign or not fatal), and others which are carcinogenic (*i.e.*, fatal).

Ramboll Environ attempted to re-create the dose-response modeling for the female mouse endpoints using the same time-to-tumor model provided in the current version of the US EPA BMD software. However, we could not completely replicate US EPA numbers. In attempting to do so, we identified several inconsistencies in the US EPA method and other issues that prevented full replication of US EPA's estimates. Furthermore, we were unable to identify adequate documentation supporting US EPA's calculations. The need for transparency highlighted by the NRC (2014), and as underscored by our inability to replicate the 2010 IUR, demonstrate the need to review and revise the IUR for chloroprene.

Examples of the inconsistencies encountered in our independent modeling of the NTP (1998) data included the following:

1. We were unable to confirm which version of the US EPA Benchmark Dose Modeling Software was used to conduct the modeling presented in the 2010 Review. This is significant because it appears that US EPA used a version of the model (from 2009) that may have contained important errors that were later corrected (personal communication with John Fox, US EPA, June 16, 2016). This could also explain some of the discrepancies in our results compared to those presented in the 2010 Review.
2. US EPA did not provide the complete input files for the model, but only a summary; therefore, we could not verify the data needed for conducting the time-to-tumor model (time of death of the animals, tumor status: censored (C) for no tumor, incidental (I) or fatal (F) tumors, or unknown (U) when there is no tissue or tissue was unusable). The lack of transparency made it difficult to verify whether US EPA conducted the modeling appropriately.
3. For the analysis of the incidence of forestomach tumors, US EPA calculated a power parameter (c), as described above, outside of the modeling program and entered it as a specific variable in the analysis. This parameter necessarily was calculated outside of the program because the program was unable to calculate it. It was unclear how US EPA calculated this parameter and whether this value is larger or smaller than what would be predicted by the program. This could impact the results and introduced additional uncertainty.
4. US EPA did not apply a consistent methodology across all the endpoints and time points that were examined. For example, in some cases animals that had no tumors or evidence that tumors were naturally "digested" by the animal (autolyzed tumors) were simply removed from the analysis (*e.g.*, for the forestomach analysis) and in other cases these were treated as "unknown" tumors (*e.g.*, in the mammary analysis). This approach would result in an overestimate of risk and there was no clear reason why US EPA took this approach.
5. There were also inconsistencies in the number of animals that were reported in each endpoint and time-point group. For example, the number of animals considered in Table C-1 of the 2010 Review (data from NTP 1998) did not match the numbers in Table 5-4 (US EPA 2010a). The major differences were identified in the total number of animals examined for tumors of the skin, mammary gland, forestomach, Harderian gland, and Zymbal's gland, and for the dose levels up to 32 ppm, depending on the endpoint. US EPA reported that tissue from 50 animals was examined, whereas NTP (1998) reported that tissue from only 49 animals was examined. Although this may not have impacted the results significantly, it indicated that US EPA allowed errors in their reporting of the results and possibly made errors inputting the results into the model, some of which might be consequential. Without full transparency and availability of model inputs, this could not be verified.

Ramboll Environ analyzed each endpoint independently, as was done by US EPA, but did not combine the estimates to obtain a composite IUR. We did not agree that US EPA's approach was standard or scientifically justified given that independence could not be confirmed and the MOA across tumor types was unknown. In addition, we corrected the issues associated with the appropriate counts and, following US EPA guidance, removed any unknowns when using an incidence-only analysis (assuming all tumors observed were incidental and were not fatal to the animals). A comparison of our independent results and those generated by US EPA is presented in Table 7.2.

Table 7.2. Comparison of Dose-Response Modeling for Female Mice at a Benchmark Response of 0.01

Site	US EPA Results from Tables C-3 and C-4							Ramboll Environ Results							
	Stage	LL	χ2	AIC	Model Selection	BMD ppm	BMDL ppm	Stage	LL	χ2	p-value	AIC	Model Selection	BMD ppm	BMDL ppm
Lung					One-stage model			3	-83.0	-0.11	0.74	176.04			
								2	-82.96	0.00	1.00	173.93			
	1	-83.02	—	172.0		0.11	0.09	1	-82.96			171.93	Lowest AIC	0.11	0.08
Hemangiomas, hemangio-sarcomas, (fatal) (highest dose group dropped)	3							3	FAILED			279.74			
	2	-135.85	5.34	279.7	χ2, lowest AIC	3.12	0.64	2	-135.87	5.34	0.02	279.74	Lowest AIC	3.04	0.47
	1	-138.52	—	283.0				1	-138.54			283.08			
Hemangiomas, hemangio-sarcomas, (all incidental) (highest dose group dropped)	3							3	FAILED						
	2	-65.81	2.28	139.6	Lowest AIC	4.61	2.02	2	-65.74	2.22	0.14	139.48	Lowest AIC	4.60	1.92
	1	-66.95	—	139.9				1	-66.85			139.70			
Harderian gland	3	-58.26	0.02	126.5				3	-58.22	0.02	0.89	126.45			
	2	-8.27	0	124.5				2	-58.23	0.00	0.98	124.47			
	1	-58.27	—	122.5	Lowest AIC	2.58	1.20	1	-58.23			122.47	Lowest AIC	2.50	1.14
Mammary gland carcinomas, adenoacanthomas	3				One-stage model			3	-84.21	0.00	1.00	178.42			
	2							2	-84.21	0.00	0.99	176.42			
	1	-87.96	—	181.9		1.95	1.34	1	-84.21			174.42	Lowest AIC	2.03	1.38
Forestomach	3	-19.17	0.84	48.35				3	-19.18	0.84	0.36	46.36			
	2	19.60	2.35	45.19	Lowest AIC	20.94	5.69	2	-19.60	2.35	0.13	45.20	Lowest AIC	20.54	5.48
	1	-20.77	—	45.54				1	-20.78			45.55			
Hepatocellular adenomas, carcinomas	3				One-stage model			3	-119.94	0.00	1.00	249.87			
	2							2	-119.94	0.00	1.00	247.87			
	1	-119.2	—	245		0.40	0.23	1	-119.94			245.87	Lowest AIC	0.39	0.23
Skin	3				One-stage model			3	-87.395	0.00	1.00	184.79			
	2							2	-87.395	0.00	0.99	182.79			
	1	-87.463	—	180.9		0.91	0.67	1	-87.395			180.79	Lowest AIC	0.89	0.67
Zymbal's gland	3	-11.402	0.65	32.8				3	-11.406	0.66	0.42	32.81			
	2	-11.726	1.77	31.45				2	-11.734	1.76	0.19	31.47			
	1	-12.611	—	31.22	Lowest AIC	15.78	5.76	1	-12.612			31.22	Lowest AIC	29.9	8.23

AIC: Akaike Information Criterion; BMD: benchmark dose; BMDL: lower 95% confidence limit of the benchmark dose; LL: log likelihood

7.7 Conclusions

US EPA applied a number of scientifically unsupported conservative assumptions in deriving the IUR for chloroprene that resulted in substantial overestimation of the IUR and added uncertainty to the toxicity estimate. Consistent with the majority of available IRIS profiles on other chemicals, the IUR should be based on the most sensitive endpoint in the most sensitive species, as this will be protective for other effects. Not assuming a systemic lesion for lung cancers yields an initial IUR of 1.06×10^{-4} based on the female mouse as the most sensitive species. In recommending a final IUR based on the mouse data, US EPA should have considered the significant pharmacokinetic differences between species and applied the PBPK model for extrapolating from animals to humans (Himmelstein *et al*. 2004), as demonstrated in Section 10.

8 THE CHLOROPRENE IUR COMPARED TO KNOWN CHEMICAL CARCINOGENS

The chloroprene IUR reported in the 2010 Review is much higher than those of similar chemicals, including known carcinogens. We compared (and summarize below) the IURs for all compounds classified by IARC as Group 1 (carcinogenic) or 2A (probably carcinogenic), which generally correspond with US EPA's classification for known or likely/probable human carcinogens. We used IARC classifications because IARC generally applied consistent methods and criteria for evaluating human carcinogens.

We also obtained the US EPA WOE classification and basis of the IUR for carcinogens for which US EPA has calculated and reported an IUR. These compounds are summarized in a table developed and updated by US EPA to be used in dose-response assessments of hazardous air pollutants.[7] In the US EPA table, all hazardous air pollutants are listed with available toxicity values based on source.

We excluded metallic compounds, which tend to be associated with particulate exposures, and mixtures, such as coke oven emissions. We sorted the remaining compounds by the IUR calculated by US EPA, from highest to lowest (Table 8.1). In addition, the table shows the WOE conclusions by IARC, the dates of each evaluation, and the relative strength of the epidemiological evidence. More detailed information on the toxicity evaluations and epidemiological evidence can be found in Appendices A and B, respectively.

[7] See Table 1 available at https://www.epa.gov/fera/prioritization-data-sources-chronic-exposure

Table 8.1. Summary of Potentially Carcinogenic Compounds by IUR Listed in IRIS

Chemical Name	US EPA WOE	Year	IARC WOE	Year	IUR per µg/m3	MOA	Basis of IUR/ Endpoint	Strength of Epidemiology Evidence
Benzidine	A	1987	1	2012	0.067	M*	Human/ bladder	Moderate
Bis(chloromethyl) Ether (BCME)	A	1988	1	2012	0.062		Rat/lung	Moderate
Nitrosodimethyl - amine (NDMA)	B2	1987	2A	1987	0.014	M*	Rat/liver	Limited
Ethylene dibromide	LH	2004	2A	1999	0.0006		Mouse/ nasal	Limited
Chloroprene	LH	2010	2B	1999	0.0005	M*	Mouse/ multiple	Limited
Acrylamide	LH	2010	2A	1994	0.0001	M*	Rat/ thyroid	Limited
Polychlorinated biphenyls	B2	1996	2A	2013	0.0001		Rat/liver	Very limited
1,3-Butadiene	CH	2002	1	2012	0.00003		Human/ leukemia	Strong (high exposures)
Formal dehyde	B1		1		0.000013		Human/nas al	Moderate (high exposures)
Vinyl chloride	CH	2010 Draft	1	2012	0.0000088		Rat/liver	Moderate (high exposures)
Benzene	CH	2003	1	2012	0.0000022 to 0.0000078		Human/ leukemia	Strong (high exposures)
Trichloroethylene	CH	2011	2A	2014	0.0000041	M*	Human/ kidney	Moderate
Epichl orohydrin	B2	1988	2A	1999	0.0000012		Rat/ kidney	Very limited
Tetrachloroethene	LH	2012	2A	2014	0.00000026		Mouse/ liver	Limited for bladder/NHL/ MM

US EPA WOE (2005 Guidelines) = CH - carcinogenic to humans; LH - likely to be carcinogenic; US EPA WOE (1986 Guidelines): A - human carcinogen; B1 - probable carcinogen, limited human evidence; B2 - probable carcinogen, sufficient evidence in animals; IARC WOE for carcinogenicity in humans (1 - carcinogenic; 2A - probably carcinogenic; 2B - possibly carcinogenic).; US EPA MOA (2005 Guidelines) M* - mutagenic and early life data lacking. NHL- non-Hodgkin lymphoma; MM – multiple myeloma

Despite being classified by IARC as a 2B carcinogen, chloroprene has the 5th highest IUR (see Table 8.1), which is orders of magnitude greater than the IURs for the known carcinogens vinyl chloride, 1,3-butadiene, and benzene. Three of the compounds with IURs higher than chloroprene (benzidine, bis(chloromethyl)ether [BCME], and N-Nitrosodimethylamine [NDMA]) have IURs that are based on reviews from the 1980s, performed before new methods were developed for integration of evidence, and likely would be different using current methods. Although there may be more recent data available to update the estimates for these compounds, two of these compounds are no longer of concern for human exposures: benzidine is no longer produced in the US (US EPA 1987a); additionally, there is very limited production of BCME, and what is produced or used is highly regulated (Bruske-Hohfeld 2009).

The only other compound with a higher IUR than chloroprene is ethylene dibromide (EDB)(US EPA 2004). US EPA (2004) described a single epidemiological st udy of occupational exposures to EDB, which was determined to be inadequate due to lack of exposure information and potential co-exposures to other carcinogens. Therefore, the IUR for ethylene dibromide was based on animal study results. Like

chloroprene, however, there were several important areas of uncertainty, including the extrapolation to low doses from high doses in rats, the application of the dose for respiratory tumors, portal of entry vs. systemic effects, and the need to account for metabolic differences between mice and humans. At the time of the assessment, a pharmacokinetic model was available (Hissink *et al*. 2000, Ploemen *et al*. 1995) but, as in the case of chloroprene, it was not deemed adequate for use by US EPA due to limited validation of the model. Therefore, updating the IUR for EDB also may be warranted. [8]

In contrast, there are several examples of carcinogenic compounds that have IURs that are *1 to 2 orders of magnitude lower* than chloroprene and for which US EPA has based the WOE evaluation and IUR development on much stronger positive human epidemiological evidence (1,3-butadiene and benzene) or for which US EPA appropriately used PBPK modeling to extrapolate results from animals to humans (vinyl chloride). In fact, one of the reasons US EPA classified chloroprene as a likely human carcinogen was structural similarities with 1,3-butadiene and vinyl chloride (US EPA 2010a), and it is particularly relevant to recognize how much higher the 2010 chloroprene IUR is compared to vinyl chloride and 1,3-butadiene. Both of these compounds were classified as known human carcinogens based on both stronger epidemiological evidence and supporting animal evidence than that available for cholorprene.

Vinyl chloride presents a relevant comparison to chloroprene based on its structural similarity to chloroprene and has been classified by IARC (2012) and US EPA (2000) as a known human carcinogen. Unlike chloroprene, however, the epidemiological evidence linking vinyl chloride with angiosarcomas of the liver, as well as primary hepatocellular cancers, is clear and consistent (Mundt *et al*. 2000, Boffetta *et al*. 2003, Mundt *et al*. 2017). US EPA appropriately applied a PBPK model for vinyl chloride to account for differences between animals and humans, resulting in a cancer IUR that is approximately 57 times lower than the IUR for chloroprene. When accounting for metabolic differences between animals and humans using a PBPK model, the cancer IUR for vinyl chloride was found to be consistent with risk estimates based on human epidemiological data and were lower than those based on external dose concentrations by a factor of 80 (Clewell *et al.* 2001).

1,3-butadiene has an extensive literature that describes its pharmacokinetics (US EPA 2002). Like chloroprene, the carcinogenetic mode of action of 1,3-butadiene is proposed to be related to its reactive metabolites, and results from PBPK models have demonstrated that there are important species differences in the rates of formation and detoxification of these reactive metabolites. In fact, the model results showed that, like chloroprene, pharmacokinetics can explain why mice are considerably more sensitive to the carcinogenic effects of 1,3-butadiene than other species, including humans. In comparing chloroprene with 1,3-butadiene, US EPA should have considered the differences observed across species that were also related to pharmacokinetics of 1,3-butadiene in deriving a chloroprene IUR, as similar differences across species have been observed for 1,3-butadiene.

[8] This is presented as a comparison for chloroprene, and is outside of the scope of our analysis.

There are other examples of recent assessments, such as that for trichloroethylene, for which US EPA appropriately applied a PBPK model to develop the IUR and for which epidemiological evidence is more robust than for chloroprene.

In summary, the comparison of the chloroprene IUR with the IURs of similar chemicals suggests that the chloroprene IUR from the 2010 Review is high even by IRIS standards, and that the chloroprene IUR should be reviewed and corrected.

9 A PBPK MODEL FOR CHLOROPRENE

9.1 PBPK modeling should be used to quantify the pharmacokinetic differences between species

PBPK modeling is used to predict the absorption, distribution, metabolism and excretion of chemical substances in humans and other animal species. These models are based on the integration of the available science for a specific compound. PBPK modeling is particularly important for use in extrapolating results from animal studies to develop toxicity values for humans, especially when there are significant differences across species. The "Guidelines for Carcinogenic Risk Assessment" (US EPA 2005) and the NRC review of the IRIS process (NRC 2014) recommend that if sufficient and relevant quantitative information is available (such as blood/tissue partition coefficients and pertinent physiological parameters for the species of interest), PBPK models should be constructed to assist in the determination of tissue dosimetry, species-to-species extrapolation of dose, and route-to-route extrapolation.

In the 2010 Review, US EPA acknowledged the shortcomings in their derivation of the chloroprene IUR, noting that: "Ideally, a PBPK model for the internal dose(s) of the reactive metabolite(s) would decrease some of the quantitative uncertainty in interspecies extrapolation; however, current PBPK models are inadequate for this purpose" (US EPA, 2010a). Although the PBPK models have been validated since the release of the 2010 Review, a PBPK model for chloroprene was available at the time US EPA prepared the 2010 Review. Despite uncertainties in the application of this model at the time of the development of the IUR, the results from these PBPK models would have explained the large observed inconsistencies in the data between mice, rats and humans. Additionally, there was substantial evidence at that time showing that external exposure concentrations from mouse chamber experiments were not representative of human health risks.

The 2010 Review noted that pharmacokinetic information on the absorption, distribution, and *in vivo* metabolism and excretion of chloroprene and/or its metabolites was available primarily for animals, but not humans. Several *in vitro* studies focused on chloroprene metabolism in lung and liver tissue fractions from rat, mouse, hamster, and humans (Cottrell *et al*. 2001; Himmelstein *et al*. 2001a, b; Himmelstein *et al*. 2004a, b; Hurst and Ali 2007; Munter *et al.* 2003; Munter *et al*. 2007; Summer and Greim 1980). These studies indicated that chloroprene is metabolized via the CYP450 enzyme system to active metabolites that are thought to be associated with the carcinogenic MOA for chloroprene. As noted in the 2010 Review, although the metabolic profile for chloroprene is qualitatively similar across species, *in vitro* kinetic studies using tissues from rodents and humans suggest significant interspecies and tissue-specific differences that, if operative *in vivo*, could account for the species, strain, and sex differences observed in chloroprene-induced *in vivo* effects.

The available *in vitro* information on the metabolism of chloroprene (Cottrell *et al.* 2001, Himmelstein *et al*. 2001b, Himmelstein *et al*. 2004a) demonstrates significant quantitative differences across species in the production of the major metabolites of chloroprene, and in particular, in the production of the epoxide likely to be the

carcinogenic constituent. The results from the *in vitro* studies indicate that greater amounts of these metabolites are produced in mice, followed by rats, and lastly in hamsters and humans. The 2010 Review discussed these differences, but did not incorporate this information when calculating the human equivalent dose for dose-response modeling. Himmelstein *et al*. (2004a) also noted species differences in the detoxification of epoxide metabolites, most notably the epoxide hydrolase, which serves to eliminate any epoxide formed. For example, the cross-species ranking of intrinsic clearance in the liver for enzymatic hydrolysis of the chloroprene metabolite was human ~ hamster > rat > mouse. In the lung, the order was human ~ hamster > rat ~ mouse. Therefore, the mouse not only had the highest capability for the generation of epoxide metabolites, but also the slowest capacity for clearance.

Overall, the balance of reactive metabolite formation and detoxification across species indicates that the mouse would be the most sensitive species, based on higher rates of epoxide formation, slower hydrolysis, and more enzyme activity. The mouse-specific pharmacokinetics all contribute to potentially increased formation and sustained concentrations of potentially toxic metabolites at lower exposures to chloroprene, explaining the increased sensitivity of this species.

The 2010 Review relied on the animal chamber air concentrations for the mouse exposure data to calculate the human IUR. Himmelstein *et al*. (2004b) demonstrated that there was no dose-response relationship when air concentrations from animal chambers (the administered dose) were used, whereas when the internal dose [9] was used (obtained from the PBPK model) a dose-response was clearly observed with relation to lung tumors. This is shown in Table 9.1, where the lung tumor incidence risk is assessed based on the internal dose. This table not only illustrates the dose-response based on internal dose, but clearly highlights the differences across species, showing that the mouse is the most sensitive species. When evaluating internal dose, which accounts for metabolic differences between mice, rats and hamsters, the differences in the lung tumor response across these species can be explained.

[9] In an experimental setting the administered dose is the concentration of the chemical that is given to the animal (measured in air, water, etc.), whereas the internal dose is the concentration of the chemical that is actually absorbed by the animal (measured inside the animal's body) and delivered to the target tissue.

Table 9.1. Exposure-Dose-Response for Rodent Lung Tumors

	Exposure concentration (ppm)	PBPK internal dose [a]	Lung tumor incidence	Number of animals	Extra risk (%)[b]
Hamster	0	0	0	100	0
	10	0.18	0	97	0
	50	0.88	0	97	0
Wistar rat	0	0	0	97	0
	10	0.18	0	13	0
	50	0.89	0	100	0
Fischer rat	0	0	3	50	0
	12.8	0.22	3	50	0.3
	32	0.55	6	49	7.7
	80	1.37	9	50	14.0
B6C3F1 mouse[d]	0	0	15	50	0
	12.8	3.46	32	50	48.3
	32	5.30	40	50	70.4
	80	7.18	46	50	89.9

(a) Internal dose - average daily mg Chloroprene metabolized/g lung tissue (AMPLU).
(b) The incidence data were corrected for extra risk equal to (Pi – Po)/(1 – Po), where P is the probability of tumor incidence in "i" exposed and "o" control animals (Himmelstein *et al*. 2004b).
(c) Male Syrian hamster and Wistar rat data from Trochimowicz *et al.* (1998).
(d) Male Fischer rat and B6C3F1 mouse data from Melnick *et al*. (1996).

9.2 US EPA calculation of the human equivalent concentration for chloroprene in the 2010 Review

All of the quantitative data necessary to refine and verify the critical metabolic parameters for the existing peer-reviewed PBPK model for chloroprene (Himmelstein *et al*. 2004b) were available at the time the 2010 Review was published and could have been applied to adjust the cancer unit risk to account for species-specific target-tissue dosimetry. Instead, the 2010 Review used the default approach and limited default assumptions described in the US EPA (1994) "Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry."

The 2010 Review assumptions included the following:

1. Lung tumors result primarily from systemic distribution, and
2. Chloroprene is a Category 3 gas according to US EPA (1994) guidelines.

Based on these assumptions, US EPA calculated the human equivalent concentration for chloroprene using the default DAF for Category 3 gases. As described by US EPA (1994), DAFs are ratios of animal to human physiologic parameters, and are based on the nature of the contaminant (particle or gas) and the target site (*e.g.*, respiratory tract) (US EPA 1994). For Category 3 gases with

systemic effects, the DAF is expressed as the ratio between the animal and human blood:air partition coefficients:

DAF = (Hb/g)A/(Hb/g)H

where:

(Hb/g)A = the animal blood:air partition coefficient

(Hb/g)H = the human blood:air partition coefficient

DAF = 7.8/4.5

DAF = 1.7

Furthermore, following US EPA guidelines (1994), US EPA used a default DAF of 1 because, as US EPA noted, "In cases where the animal blood:air partition coefficient is higher than the human value, resulting in a DAF>1, a default value of 1 is substituted (US EPA, 1994)." This was a conservative assumption, as it is noted in the guidelines that the available data for rats indicated that (Hb/g)A is greater than (Hb/g)H for most chemicals. This restricted the evaluation to equivalence between the mouse and the human and did not address the important pharmacokinetic differences in chloroprene metabolism in the mouse compared to the human.

9.3 The Allen et al. (2014) study shows that a validated PBPK model should be used to update the 2010 chloroprene IUR

Allen *et al.* (2014) combined the results from the most recent PBPK models for chloroprene (Yang *et al.* 2012) with a statistical maximum likelihood approach to test commonality of low-dose risk across species. Using this method, Allen *et al.* (2014) evaluated the difference between risk estimates obtained using external (chamber air concentrations) and internal dose (calculated with the PBPK model) metrics. The PBPK model for chloroprene incorporates data regarding species differences in metabolism of chloroprene, and allows species-specific estimation of internal exposure metrics, specifically the amount of chloroprene metabolized per gram of lung tissue. By using this model, IURs can then be compared across species based on equivalent internal exposure metrics rather than external air concentrations measured outside of the body. This is an important consideration when the toxicity of a compound is related to how the compound is metabolized in animals vs. humans.

Allen *et al.* (2014) found that for chloroprene, external concentration-based estimates were not appropriate for calculating and comparing cancer risks across species. As discussed in Section 5, epidemiological studies related to occupational exposures to chloroprene must also be considered in evaluating the unit risk estimate. These epidemiological studies provide little or no scientific support for the hypothesis that human and animal low-dose risks were equivalent when expressed as a function of air concentrations. In contrast, by accounting for the daily amount of chloroprene that is metabolized per gram of tissue at the target site for different species, the PBPK results provided a substantially better fit of the models to the data. Importantly, the differences in internal dose across species explained the greater sensitivity in mice (Himmelstein *et al.* 2004b), as well as the lower sensitivity of humans.

Allen *et al*. (2014) derived cancer unit risks for respiratory system cancer using the PBPK model results from both animal and human data that ranged from 2.9×10^{-5} to 1.4×10^{-2} per ppm (8.1×10^{-9} to 3.9×10^{-6} per µg/m^3), with a maximum-likelihood estimate of 6.7×10^{-3} per ppm (1.86×10^{-6} per µg/m^3). This estimate is about 100 times lower than the 2010 Review estimate of 6.5×10^{-1} per ppm (1.81×10^{-4} per µg/m^3) based on the incidence of lung tumors in female mice. It is also important to note that the Allen *et al*. (2014) assessment is highly conservative in that it does not account for species-to-species differences in detoxification and pharmacodynamics, which is justified and would lead to an even lower IUR.

It is difficult to apply the method used by US EPA for multi-tumor adjustment using the data provided in the Allen *et al*. (2014) publication, because the Allen *et al*. data were limited to lung tumors . However, this method likely would generate an estimate that is 100 times lower than the US EPA estimate. A similar rationale can be used for the application of the ADAF, yielding an IUR of approximately 5×10^{-6} per µg/m^3. However, because there is limited evidence for mutagenicity, we concluded that the 2010 IUR should be closer to the estimate calculated by Allen *et al*. (2014) of 1.86×10^{-6} per µg, and that this value is appropriately protective.

Overall, the evidence indicates that humans are far less sensitive to chloroprene exposures than mice, which is also consistent with the lack of clear or consistent epidemiological evidence of carcinogenicity as discussed in Section 5.

10 CALCULATION OF AN UPDATED CHLOROPRENE IUR

Ramboll Environ recalculated the IUR for chloroprene using the same standard methodologies that US EPA has employed in IRIS assessments for several known carcinogens, but did not employ in the 2010 Review of chloroprene. Ramboll Environ employed this methodology to reduce the significant uncertainty associated with extrapolating results from animal experiments to humans (and from one route of exposure to another), and in consideration of the substantial body of evidence demonstrating large differences in sensitivity to chloroprene across species. These differences reflect underlying pharmacokinetic differences that, if not taken into account, result in a highly inflated IUR value such as that derived in the 2010 Review.

The Allen *et al*. (2014) analysis provided a rigorous approach for integrating the available epidemiological and toxicological evidence to estimate a chloroprene IUR. However, it incorporated a maximum likelihood statistical method different from the traditional PBPK models used by US EPA in estimating IURs and other toxicity values, such as reference concentrations (RfC) or reference doses (RfD). In deriving an IUR, US EPA typically applies a PBPK model to estimate an internal dose at the target organ of interest (*e.g.*, the lung), based on the mode of action.

As discussed above, it is hypothesized that chloroprene itself does not exert a carcinogenic effect, but rather a metabolite of chloroprene exerts the effect. Therefore, carcinogenicity depends on the internal concentration of the metabolite, and not the internal (or external) concentration of chloroprene. The internal concentration of the metabolite is determined by how rapidly it is produced and eliminated from the body, and metabolite production and elimination rates vary considerably across species. Therefore, accounting for species-specific pharmacokinetic differences using PBPK modeling is critical. The US EPA (2005) Guidelines for Carcinogen Risk Assessment states that PBPK models

> *"…generally describe the relationship between exposure and measures of internal dose over time. More complex models can reflect sources of intrinsic variation, such as polymorphisms in metabolism and clearance rates. When a robust model is not available, or when the purpose of the assessment does not warrant developing a model, simpler approaches may be used."*

The preferred approach to PBPK modelling has been documented in the US EPA (2005) "Guidelines for Carcinogen Risk Assessment." Furthermore, US EPA has applied these PBPK models in estimating toxicity values for several compounds; for example, dichloromethane, vinyl chloride, tetrachloroethylene, carbon tetrachloride, and acrylamide, specifically to reduce uncertainty associated with animal-to-human extrapolation or route-to-route extrapolation. Although there may be no "perfect" model, toxicity values derived from models that best reduce uncertainty are more scientifically supportable and therefore preferred to those obtained using default adjustment factors (DeWoskin *et al*. 2007).

When an IUR is based on animal data, an animal PBPK model is required to estimate the internal dose corresponding to each of the administered

concentrations (*i.e.*, ppm in the chamber air), following the same pattern of exposure of the animals in the study (*e.g.*, days/week). This internal dose estimate is then used (instead of the air concentration) for dose-response modeling and estimating a Point of Departure (POD). This POD corresponds to the internal dose in the animal. The human PBPK model then is applied to account for known physiological and metabolic differences between the animal and human. This is accomplished by estimating the equivalent external concentration that results in the internal dose equal to the POD derived from the animal data. The IUR is estimated by dividing the risk level (benchmark risk or BMR associated with the POD) by the POD. The IUR is interpreted as the risk per unit (ppm or $\mu g/m^3$) intake.

Chloroprene PBPK modeling results for mice, rats, and humans are reported in Yang *et al*. (2012). Specifically, the internal dose estimates associated with the concentrations administered to both mice and rats in the NTP (1998) study are provided, including gender-specific internal tissues doses, *i.e.*, the average amount of chloroprene metabolized per day per gram of lung (AMPLU) based on the PBPK model. These internal doses represent the concentration of the toxic moiety (*i.e.*, the chloroprene metabolite) identified by US EPA as the key carcinogenic metabolite (US EPA, 2010a). The Yang *et al*. (2012) analysis showed that mice had the greatest amount of chloroprene metabolized per gram of lung, followed by rats and then humans. The human and rat showed linear dose-responses over the range of NTP bioassay concentrations of 12.8, 32 and 80 ppm. Based on this, the following was established as the relationship between the internal dose and the external exposure (ppm) in the human: 1 ppm of constant external exposure in the human results in 0.008 µmole of chloroprene metabolized per gram of lung tissue per day.

We relied on the internal dose results from the PBPK modeling conducted and reported by Yang *et al*. (2012), consistent with the PBPK modeling approach that US EPA has used in other IRIS assessments (dichloromethane, vinyl chloride, tetrachloroethylene, carbon tetrachloride). In addition, also consistent with the conclusions in the US EPA (2010) chloroprene review regarding the most sensitive endpoint in the most sensitive species, we estimated the chloroprene IUR using the results for the combined incidence of alveolar/bronchiolar adenomas and carcinomas (the most sensitive endpoint) in female mice (the most sensitive species and gender).

Using the internal doses for female mice as provided in Table 5 of Yang *et al.* (2012) (see Table 10.1), time-to-tumor modeling of the lung alveolar/bronchiolar adenomas and carcinomas was performed using the Multistage-Weibull model provided with the US EPA BMDS software (February 25, 2010 version). Time-to-tumor dose-response modeling is preferred and was used in the US EPA (2010) chloroprene assessment to model the incidence of tumors from the NTP (1998) bioassay. This type of dose-response model was necessary, as the survival of the female mice exposed to chloroprene was "significantly less than that of the chamber control" (NTP 1998). Time-to-tumor models adjust for early death of the animal, and thus the probability that the animal, if it had lived longer, may have developed the tumor of interest.

The female mouse data that we used in our analyses are presented in Table 10.2, with each animal's time of death and the observation of C, I, F or U to indicate: C=censored or the animal did not have the tumor of interest; I = incidental or the animal had the tumor of interest but it was not indicated as the cause of death; F=fatal or the animal had the tumor of interest and it was indicated as the cause of death; or U=unknown or the presence of the tumor could not be determined as the organ was autolyzed or missing in the animal. The alveolar/bronchiolar adenomas or carcinomas were all considered to be incident tumors, consistent with the time-to-tumor dose-response models and approaches used in US EPA (2010). One tumor was classified as unknown in one animal in the 12.8 ppm group, so modeling was conducted both including and excluding that animal to determine if there was any major impact on the outcome of the dose-response modeling.

Consistent with the US EPA (2010) approach, we selected a benchmark risk (BMR) of 1% (see Table 10.3 and Appendix C for the complete Multistage-Weibull modeling results). Note that models including or excluding the animal with the unknown tumor (Animal # 320)[10] generated the same estimated IUR. We calculated the external human dose (in ppm) by dividing the POD or lower bound on the benchmark dose (BMDL) by the factor of 0.008 to obtain the external concentration for continuous exposure in the human in ppm associated with the internal POD. We then calculated t he IUR by dividing the BMR by the human equivalent POD/BMDL in either ppm or µg/m^3:

The final results are presented in Table 10.4. Using the standard methods applied in other IRIS assessment by US EPA and publically available publish ed data, the recalculated IUR for chloroprene was 1.1x 10^{-2} per ppm or 3.2 x 10^{-6} per µg/m^3. This result, which incorporates appropriate PBPK models and adjustments necessary to extrapolate the findings from animal studies to relevant human exposure considering the differences in pharmacokinetics , is consistent with methods used in other IRIS assessments by US EPA. However, the IUR value is very different from that recommended in the 2010 Review and underscores the scientific importance of correcting and updating it.

[10] When it cannot be determined if an animal had the tumor of interest due to the organ being missing or deteriorated too much to examine, the animal will get an observation of "unknown". This data can be used in a time-to-tumor model (*e.g.* Multistage Weibull) as a time of death is available for that animal. In this case, including the animal with an observation of unknown or excluding the animal from the modeling did not result in a detectable difference in the results.

Table 10.1. Internal and External Doses from Yang et al. (2012)

External Dose (ppm)	PBPK Internal Dose Metric[11] (µmole CD metabolized /gram lung tissue/day)		Linear Relationship between ppm and PBPK metric in humans
	Mouse	Human	
12.8	0.74	0.1	0.008
32	1.19	0.25	0.008
80	1.58	0.64	0.008

[11] Data from Yang *et al.* (2012) Table 5.

Table 10.2. NTP (1998) Study – Female B6C3F$_1$ Mice Lung Alveolar/bronchiolar adenoma or carcinoma

Control = 0 ppm			Dose = 12.8 ppm			Dose=32 ppm			Dose = 80 ppm		
0 µmole/g tissue/day			0.74 µmole/g tissue/day			1.19 µmole/g tissue/day			1.58 µmole/g tissue/day		
Animal #	Time (wks)	Obs.[12]	Animal #	Time (wks)	Obs.	Animal #	Time (wks)	Obs.	Animal #	Time (wks)	Obs.
141	5	C	318	41	C	505	31	C	738	1	C
110	69	C	330	46	C	532	50	I	711	36	C
138	70	C	350	46	U	545	54	C	725	47	I
107	71	C	311	63	C	535	56	C	734	48	C
130	76	C	321	64	I	540	57	C	729	55	C
135	78	C	342	69	C	530	61	C	721	64	C
126	88	C	303	75	I	502	63	I	705	65	I
105	91	C	327	76	C	548	65	I	741	66	I
146	91	C	344	78	C	510	67	C	701	67	C
124	95	C	315	79	C	529	68	C	716	67	I
133	97	C	316	79	C	521	70	C	735	70	I
103	98	C	328	79	C	506	72	I	709	75	I
127	101	C	301	87	C	512	72	I	717	75	I
132	101	I	324	89	I	524	73	C	722	75	I
101	105	C	347	89	I	523	74	I	749	75	I
102	105	C	304	90	C	531	75	I	715	76	I
104	105	C	325	91	I	547	75	C	726	76	I
106	105	C	343	91	I	518	76	I	745	77	C
108	105	C	349	91	C	519	76	I	740	79	I
109	105	C	313	97	C	503	77	C	710	81	I
111	105	C	314	97	I	504	77	I	702	83	I
112	105	C	329	97	I	511	78	C	704	83	I
113	105	C	310	98	I	528	79	I	746	83	I
114	105	C	308	99	C	546	79	I	714	84	I
115	105	C	319	99	I	533	82	I	730	86	I
116	105	C	323	99	I	520	84	I	703	87	C
117	105	C	332	99	I	522	84	C	713	88	I
118	105	C	340	99	I	536	86	I	728	88	I
119	105	C	345	100	C	507	87	I	712	90	I
120	105	C	306	101	I	525	87	C	737	90	I

[12] Observation s are coded as C=censored, the animal did not have the tumor of interest
I = Incidental, the animal had the tumor of interest but it did not cause death
F = fatal, the animal had the tumor of interest and it was the cause of death (none in this dataset)
U = Unknown, it is not known if the animal had the tumor or not due to organ being autolyzed or missing

Control = 0 ppm			Dose = 12.8 ppm			Dose=32 ppm			Dose = 80 ppm		
0 µmole/g tissue/day			0.74 µmole/g tissue/day			1.19 µmole/g tissue/day			1.58 µmole/g tissue/day		
Animal #	Time (wks)	Obs. [12]	Animal #	Time (wks)	Obs.	Animal #	Time (wks)	Obs.	Animal #	Time (wks)	Obs.
121	105	C	334	102	I	526	87	I	718	91	I
122	105	C	346	102	I	527	89	I	727	91	I
123	105	I	331	103	C	539	89	I	732	91	I
125	105	C	341	103	I	541	90	I	733	91	I
128	105	C	302	105	I	542	90	I	736	91	I
129	105	C	305	105	I	544	90	I	747	91	I
131	105	I	307	105	I	501	91	I	750	91	I
134	105	I	309	105	C	509	91	I	724	92	I
136	105	C	312	105	C	516	91	I	742	93	I
137	105	C	317	105	I	537	92	I	748	93	I
139	105	C	320	105	I	508	93	I	707	94	I
140	105	C	322	105	I	517	94	I	708	95	I
142	105	C	326	105	C	538	94	I	739	95	I
143	105	C	333	105	C	550	94	I	744	96	I
144	105	C	335	105	I	534	96	I	723	97	I
145	105	C	336	105	I	549	96	C	731	97	I
147	105	C	337	105	I	513	97	I	743	98	I
148	105	C	338	105	C	515	99	C	706	105	I
149	105	C	339	105	I	543	103	I	719	105	I
150	105	C	348	105	I	514	105	I	720	105	I

Table 10.3. Multistage -Weibull Time-to-Tumor Modeling Results for a Benchmark Risk of 1%

Site	Stages	Log-Likelihood	AIC	Model Selection	BMD (µmole/gram lung tissue/day)	BMDL (µmole/gram lung tissue/day)	BMDU (µmole/gram lung tissue/day)
Female Mouse Lung – incidental. Animal with unknown status excluded	3	-82.607	175.21		0.0098	0.0052	0.0783
	2	-82.669	173.34	Lowest AIC	0.0677	0.0069	0.0770
	1	-85.722	177.44		0.0049	0.0039	0.0060
Female Mouse Lung – incidental. Animal with unknown status included	3	-82.674	175.35		0.0099	0.0053	0.0791
	2	-82.739	173.48	Lowest AIC	0.0676	0.0070	0.0768
	1	-85.882	177.77		0.0048	0.0037	0.0060

Table 10.4. Calculation of IURs using Human Equivalent Concentrations

Results from 2-stage Multistage Weibull Time-to-tumor model	BMR = 0.01				
	BMDL (µmole/gram lung tissue/day)	External Concentration (ppm) [13]	IUR (per ppm)	External Concentration (µg/m^3)	IUR (per µg/m3)
Female Mouse Lung – incidental. Animal with unknown status excluded	0.0069	0.863	0.012	3122	3.2E-06
Female Mouse Lung – incidental. Animal with unknown status included	0.0070	0.875	0.011	3168	3.2E-06

[13] Human doses in ppm are obtained by dividing the BMDL by the conversion factor derived from Yang et al. (2012) Table 5 of 1 ppm = 0.008 µmole/gram lung tissue/day

11 CANCER RISK ASSESSMENT: VALIDATION OF THE CHLOROPRENE IUR

As a validity check, we calculated the excess cancers that would be expected based on application of the US EPA IUR at the chloroprene exposure concentrations reported by Marsh *et al*. (2007b). Marsh *et al*. (2007b) modeled the chloroprene exposures for all unique job title classes using six exposure classes for each plant over the entire period of chloroprene production in each plant. Job title classes and time-specific chloroprene exposure estimates were linked to each worker's job history to construct a profile. These subject-specific profiles were then used to compute the statistical estimates of worker exposures used in the risk calculations presented in Table 11.1.

As shown in Table 11.1, we calculated risk estimates (excess cancers) for each of the unit risk estimates that US EPA derived for chloroprene in the 2010 Review. These included an IUR based on lung tumors, an IUR based on multiple tumors, and an IUR adjusted for lifetime exposures (with application of the ADAF). In addition, we calculated cancer risk estimates based on the IUR derived by Allen *et al*. (2014), as well as the IUR provided in this report, both of which account for pharmacokinetic differences between animals and humans. We derived risk estimates using exposure estimates from the Louisville plant (Marsh 2007a, b), as these exposures were much higher (at least an order of magnitude or more) than the exposures at other plants. In Table 11.1, we compared calculated excess cancer risk estimates with the excess liver cancers observed at the Louisville plant (observed cases minus expected cases, based on both US and local county rates).

The risk assessment summarized in Table 11.1 illustrates that cancer risk estimates calculated based on the IUR in the 2010 Review overestimated actual liver cancer risks. Marsh *et al*. (2007a) reported less than one excess liver cancer death when compared to US rates, and a deficit of about two liver cancer deaths when compared to the more appropriate local country rates. In contrast, using the 2010 Review IUR and mean reported chloroprene exposures, approximately 15 excess liver cancer deaths should have been observed. Repeating this exercise using the risk estimate derived by Allen *et al*. (2014), as well as the Ramboll Environ estimated IUR in this report, we showed that the estimated excess cancer risk estimates were consistent with the observed cases reported by Marsh *et al*. (2007a).

Table 11.1. Cancer Risk Estimates Based on US EPA and Allen et al. (2014) IURs for Chloroprene Compared with Excess Cancers Observed in the Louisville Plant

Source	Unit risk (per ppm)	Exposure (ppm)[a]			Excess Cancers (Risk Estimate)[b]			Excess Liver Cancers (Observed-Expected)[c]	
								Comparison Group	
		Median	Mean	Max	Median	Mean	Max	US	Local County
US EPA (2010)									
lung tumor	0.65	5.23	8.42	71	3.40	5.5	46		
multi tumor	1.08	5.23	8.42	71	5.65	9.1	77		
w/ADAF	1.80	5.23	8.42	71	9.41	15.2	128	0.65	-1.89
Allen et al. (2014)									
lung tumor	0.0067	5.23	8.42	71	0.04	0.1	0.5		
Ramboll Environ									
lung tumor	0.011	5.23	8.42	71	0.06	0.1	0.8		

a Data from Marsh *et al*. 2007b (Table 3)
b Excess cancer risk calculated by multiplying the unit risk (per ppm) by the exposure level (in ppm)
c Data obtained from Marsh *et al*. 2007a (Table 3). Expected cancers = Observed/SMR

This analysis demonstrates that the 2010 Review IUR overestimates risk, and that a PBPK adjustment provides a better fit to the best available human data.

12 THE CHLOROPRENE RFC

A reference concentration (RfC) is a health risk value that is intended to be protective of non-cancer risks from inhalation in humans. The RfC reported in the 2010 Review for chloroprene is 2×10^{-2} mg/m^3. The RfC is an estimate of the daily exposure to human populations, including susceptible groups such as children and the elderly, which is considered to be without an appreciable risk for non-cancer health effects over a lifetime. The value is calculated by first determining the point of departure, traditionally using a no-observed-adverse-effect level or lowest-observed-adverse-effect level (NOAEL or LOAEL, respectively) and more recently using dose-response modeling.

Like the calculation of the cancer IUR, US EPA relied upon the results from the 2-year chronic inhalation study conducted in rats and mice by the National Toxicology Program (NTP 1998) as the basis for the RfC, but focusing on the non-cancer effects. US EPA also considered a second study conducted in a different strain of rats and in hamsters (Trochimowicz *et al.*, 1998), but did not rely on this study because it reported a high mortality rate in animals in the lowest exposure group due to failure in the exposure chamber. However, though significant histopathological lesions were reported in the NTP (1998) study in the lungs and spleen in the lowest exposure group (12.8 ppm) in B6C3F1 mice, comparatively few histopathological lesions were observed even in the highest exposure groups in Wistar rats and Syrian hamsters (Trochimowicz *et al.*, 1998).

From the NTP (1998) study, US EPA selected all the non-cancer endpoints that were statistically significantly increased in mice and rats at the low and mid-exposure levels (12.8 and 32 ppm) compared with controls. These endpoints included both portal of entry and systematic lesions observed in the nose, lung, kidney, forestomach, and spleen in mice and in the nose, lung and kidney of the rats (see Table 5-1 in US EPA 2010a). US EPA used their own benchmark dose modeling software (BMDS) to estimate a Point of Departure (POD). As with the cancer endpoints, these results suggested significant cross-species and strain differences in the toxicological response to inhaled chloroprene. In addition, for some of the endpoints, no model provided an adequate fit to the data, suggesting external concentrations may not correspond to the observed incidences. These results also underscore the importance of understanding the difference in pharmacokinetics across species to derive the most biologically relevant human equivalent RfC. PBPK methods have been used to derive appropriate RfCs for other relevant chemicals, including vinyl chloride (Clewell 2001, US EPA 2000).

The last source of uncertainty that US EPA should have considered in the derivation of the RfC is the application of uncertainty factors to the POD. US EPA applied a total uncertainty factor of 100 to the POD of 2 mg/m^3. A standard uncertainty factor of 10 was applied to account for variation in the susceptibility among members of the human population. An uncertainty of 3 was applied to account for extrapolation of animals to humans; however, this uncertainty can be removed if a validated PBPK model is used to derive a human equivalent exposure to chloroprene that accounts for pharmacokinetic differences between animals and humans. Lastly, an uncertainty factor of 3 was applied to account for database

deficiencies related to reproductive toxicity. This adjustment is also not needed based on several lines of evidence. First, chloroprene is not expected to accumulate in tissues such that in a multigenerational study, exposures to the second generation (F2) would be greater than experienced by the first generation (F1). Second, the results of a single generation reproductive toxicity study for a structurally similar chemical, 2,3-dichloro-1,3-butadiene (Mylchreest *et al*. 2006) indicate that effects at the point of contact (nasal effects) in parental animals are more sensitive than reproductive/developmental effects. Specifically, this study reported a NOAEL of 10 ppm for nasal effects in rats, and a NOAEL of 50 ppm for reproductive toxicity (changes in maternal and fetal body weights). Similarly, an unpublished one-generation reproductive toxicity study of chloroprene in rats reported a NOAEL of 100 ppm for reproductive toxicity (Appelman and Dreef van der Meulan 1979). All of these NOAELs are considerably higher than any other non-cancer effect and suggest that the application of an uncertainty factor for database deficiencies for the lack of a two-generation reproductive study is not necessary.

13 CONCLUSIONS

The IUR derived in the 2010 Report did not address the large recognized differences in cancer susceptibility across animal species, and especially between female mice and humans. Failure to apply well-accepted and now specifically validated methods for accounting for these differences led to an invalid (and implausible) IUR for chloroprene.

Our critical review and synthesis of the available evidence from toxicological, mechanistic, and epidemiological studies, as well as an integration of the evidence across these lines of scientific inquiry, determined that the approach US EPA used to derive an IUR for chloroprene relied on several unsubstantiated assumptions and failed to take into account the large inter-species cancer susceptibilities. We demonstrated that an IUR derived today would be considerably different from the one recommended in the 2010 Review. Our approach comported with US EPA methods and guidance, as well as the recommendations made by multiple NRC Committees evaluating the US EPA IRIS evaluation methods.

Although animal studies provided a positive response for carcinogenicity, the current science for chloroprene demonstrates major differences in species-specific cancer response to chloroprene exposure. Quantitative differences in pharmacokinetics across species, specifically related to differences in metabolism and detoxification of potentially active metabolites, can and should be incorporated into a corrected IUR or other risk number. In the 2010 Review, the available chloroprene pharmacokinetic findings were not incorporated to quantitatively account for differences between the mouse, rat, and human. When genotoxicity/genomics, MOA, and pharmacokinetic data are considered in an appropriately integrated manner, the data strongly suggest that the cancer responses from chloroprene are largely confined to—and possibly unique to—the female mouse. Because of these strong interspecies differences, use of the female mouse data for risk evaluation, in the absence of affirmative epidemiologic al data that can be used quantitatively, must incorporate tissue-specific dosimetry and metabolic differences. Additionally, because the available evidence does not support a mutagenic MOA for chloroprene, the cancer unit risk should not be adjusted to account for potential risks from early-life exposures with the application of the ADAF. While appropriate PBPK models were available to US EPA at the time of the 2010 Review, US EPA stated that published data were unavailable to validate the model. Data have now been published, have validated the PBPK model, and should be used to correct the IUR.

Our critical review and synthesis of all epidemiological studies of chloroprene-exposed workers, using standard methods that consider study quality and potential sources of bias, indicated no clear or consistent association between occupational chloroprene exposure and mortality from lung or liver cancers. The strongest study, in fact, demonstrated small deficits in lung and liver cancer mortality among chloroprene-exposed workers (Marsh 2007a, b). Nevertheless, in the 2010 Review, this study is cited as providing support for a causal association, directly contradicting our conclusions as well as the study authors' own conclusions. In fact, the epidemiology was consistent with the application of a PBPK model to

adjust the animal experimental evidence and account for the large differences in inter species cancer susceptibilities. There is a substantial body of evidence supporting the conclusion that humans are far less susceptible to the potential carcinogenicity of chlor oprene than mice primarily because the way humans metabolize chloroprene does not lead to the production of significant concentrations of the carcinogenic metabolite. The epidemiological study results also support this conclusion.

Using standard methods consistent with the NRC recommendations and EPA Guidelines, and the most current scientific evidence, we derived an IUR for chloroprene that is 156 times lower than that derived by US EPA. Following methods used in other IRIS assessments, we derived an IUR of 3.2×10^{-6} per $\mu g/m^3$. We request that US EPA re-evaluate and correct the IUR, which is based on the most sensitive species and endpoint (lung tumors in female mice) and apply a PBPK model to more appropriately account for the large differences between mice and humans. We recommend no further adjustment for multiple tumor sites, and no adjustment for a mutagenic MOA. Similarly, the chloroprene RfC will need to be updated to incorporate the same pharmacokinetic differences across species.

Based on a comprehensive evaluation and integration of the published epidemiological, toxicological and mechanistic evidence, we consider the US EPA 2010 Review of chloroprene to be outdated and invalid. Accordingly, US EPA should also revisit the cancer classification for chloroprene and provide a transparent and accurate narrative that reflects a weight of evidence approach. Most importantly, however, the IUR derived in the 2010 Report is not scientifically defensible and needs to be corrected.

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APPENDIX A
TOXICOLOGICAL SUMMARY OF CARCINOGENIC COMPOUNDS

Toxicological Summary of Carcinogenic Compounds

Chemical	IUR (per µg/m³)	US EPA WOE/ Year	Human Data	Animal Data	Geno-toxicity	Extrapolation Method	Species	Endpoint	Model Used	PBPK Model
Benzidine**	0.067	A/1987	Sufficient	Limited *via* inhalation	Yes	One-hit with time factor, extra risk	Human Occupational (Inhalation)	Bladder tumors	--	No
Bis(chloromethyl)ether (BCME) **	0.062	A/1988	Sufficient	Sufficient	Yes	Linearized multistage, extra risk	Rat	Respiratory tract tumors	--	No
N-Nitrosodimethylamine (NDMA **)	0.014	B2/1987	Limited due to exposure to mixtures	Limited evidence *via* inhalation	Yes	Weibull, extra risk	Rat	Liver tumors	--	No
Ethylene Dibromide	0.0006	B2/2004	Inadequate	Sufficient	Yes	Multistage	Rat	Nasal cavity tumors	Multistage-Weibull time-to-tumor	No
Chloroprene	0.0005	B1/2010	--	Clear evidence	Yes - Metabolites	Linear low-dose extrapolation	Mice	All tumor sites reported	Multistage-Weibull time-to-tumor	No
Acrylamide	0.000147	B2/2010	Inadequate	Sufficient	Yes	Route-to-route extrapolation of the oral POD	Rat	Thyroid tumors	Multistage-Weibull Time-to-tumor	No
Polychlorinated biphenyls (under reassessment)#	0.0001	B2/1996	Inadequate	Sufficient	--	Linear extrapolation below LED10s	Rat	Liver tumors	--	No
1,3-Butadiene	0.00003	A/2002	Sufficient	Sufficient	Yes - Metabolites	Linear extrapolation	Human	Leukemia	Relative Rate Model	No

Chemical	IUR (per µg/m³)	US EPA WOE/ Year	Human Data	Animal Data	Geno-toxicity	Extrapolation Method	Species	Endpoint	Model Used	PBPK Model
Formaldehyde	0.00066	Supports carcino - genicity/ 2010 (Draft)	Supportive, but alone not sufficient	Strong support	Data suggests genotoxicity	Linear extrapolation from the POD	Human	Nas o-pharynge al cancer, Hodgkin lymphoma and leukemia	--	Yes
Vinyl Chloride	0.0000088	A/2000	Sufficient	Sufficient	Yes - Metabolites	Linearized multistage method	Rat	Liver tumors	Linearized Multistage Model	Yes
Benzene	0.000002 – 0.0000078	A/2003	Strong evidence	Limited evidence	Suggestive but not conclusive	Low-dose linear; maximum likelihood	Human	Leukemia	--	No
Trichloroethylene (TCE)	0.0000041	CH/2011	Modest	Clear evidence	Data suggests potential for genotoxicity	Linear low dose-extrapolation	Human	Kidney cancer; Non - Hodgkin's lymphoma ; Liver cancer	Weighted linear regression model	No
Epichlorohydrin	0.0000012	B2/1988	Inadequate	Sufficient	Suggestive	Linearized multistage procedure, extra risk	Rat	Kidney lesions	--	No
Tetrachloroethene	0.00000026	LH/2012	Evidence of association	Evidence of association	Insufficient	Linear extrapolation	Mouse	Liver tumors	Multistage model	Yes

US EPA WOE (2005 Guidelines) = CH - carcinogenic to humans; LH - likely to be carcinogenic; US EPA WOE (1986 Guidelines): A - human carcinogen; B1 - probable carcinogen, limited human evidence; B2 - probable carcinogen, sufficient evidence in animals

* Draft version available – currently under public comment

** Only an IRIS Summary was available, not a full ToxProfile

The draft reassessment is currently in the scoping and problem formulation portion. Therefore, no updated assessment has been performed.

PBPK: physiologically based pharmacokinetic (model)

IUR: inhalation unit risk

APPENDIX B
SUMMARY OF EPIDEMIOLOGICAL EVIDENCE OF KNOWN OR LIKELY CARCINOGENIC COMPOUNDS CLASSIFIED BY US EPA

Summary of the Epidemiological Evidence of Chemical Carcinogens Classified as Known or Likely Human Carcinogens by IARC and/or US EPA

Compound	Sources	Outcomes with strong evidence	Types of studies	Quantification (if possible)	Conclusion
Benzidine	US EPA 1987a; Meigs et al. 1986; Tomioka et al. 2016; Golka et al. 2004; IARC 2012	Bladder and lung cancer	Several occupational epidemiology studies from the 1980s to 2000s for bladder cancer; 23 retrospective cohort studies from 1970s-2010s for lung cancer	SIR (bladder cancer) = 3.43, 95% CI: 1.48-6.76; (Meigs et al. 1986, cited in US EPA) Pooled risk estimate (lung cancer) = 2.33, 95% CI 1.31-4.14 (Tomioka et al. 2016) based on meta-analysis of 23 cohort studies of highly exposed workers 30-fold to 75-fold higher risk of bladder cancer based on occupational cohort studies in China 1980s –2000s (Golka et al. 2004)	US EPA: Category A; IARC 2012: Group 1, "Benzidine causes cancer of the urinary bladder." Risk of lung cancer is statistically significantly elevated; but confounding by co-exposure with beta-naphthylamine cannot be ruled out. (Tomioka et al. 2016) "Toxicologically, benzidine has been the most important carcinogenic aromatic amine directed towards the human bladder." (Golka et al. 2004)
Bis (chloromethyl) ether (BCME)	US EPA 1988a; IARC 2012; Bruske-Hohfeld 2009	Lung cancer	Occupational epidemiology studies from the 1970s-1990s	"Among heavily exposed workers, the RRs are tenfold or more." (Bruske-Hohfeld 2009)	US EPA: Category A; IARC: Group 1
Nitrosodimethylamine (also N-Nitrosodimethylamine)	US EPA 1987b; ATSDR 1989; IARC 1978	None specified in humans Numerous multisite tumors in various animal species (inhalation and oral exposures)	Animal studies of oral exposure from 1970s-1980s; two studies of inhalation exposure in animals from 1967 No studies of inhalation and cancer in humans; confounding by co-exposure cannot be ruled out	No risk estimates in humans available	US EPA – Category B2; IARC – Group 2A

Compound	Sources	Outcomes with strong evidence	Types of studies	Quantification (if possible)	Conclusion
Ethylene dibromide (also 1,2-Dibromoethane)	US EPA 2004; IARC 1999	None in humans. In animals, inhalation (long term) is associated multi-site tumors	Three occupational epidemiological studies evaluated by US EPA deemed to be inadequate	No risk estimates in humans available	US EPA - Category LH ; IARC - Categor y 2A "*inadequate evidence* in humans" but "*sufficient evidence"* in experimental animals
Acrylamide	US EPA 2010b; Pelucchi et al. 2011; IARC 1994	Little evidence in humans In animals, oral exposure associated with multi-site tumors	5 retrospective and prospective cohort studies of occupational exposure (inhalation/dermal) from the 1980s to the 2000s – no strong associations. Meta-analysis of occupational (inhalation/dermal) exposure found positive, but no statistically significant associations (Pelucchi et al. 2011)	Select SMRs (95% CI) of meta-analysis (Pelucchi et al. 2011): Pancreas, high exposure: 1.67 (0.83-2.99) Kidney, high exposure: 2.22 (0.81-4.84)	US EPA: Group B2; IARC: Group 2A (Inadequate evidence in humans; sufficient evidence in animals).
Polychlorinated biphenyls (PCBs)	US EPA 1996; ATSDR 2000; Zani et al. 2013; IARC 2016	Melanoma Inconsistent findings for non-Hodgkin lymphoma, breast cancer	Many occupational cohort studies of PCB exposure, 1980s-2010s; limitations include small sample sizes, confounding exposures, and short follow-up.	Occupational exposures SMR for melanoma = 2.4, 95% CI: 1.1-4.6 (Ruder 2006, as reported by Zani et al. 2013) RR = 4.8, 95% CI: 1.5-15.1 for high exposures (Loomis et al. 1997)	US EPA – Category B2 IARC - Group 1 Sufficient evidence for melanoma. For occupational exposures, "weak evidence of a major role of PCBs as human carcinogens" (Zani et al. 2013)
1,3-Butadiene	US EPA 2002; IARC 2008	Lymphatic and hematopoietic cancers	Many occupational cohort studies; stronger evidence of leukemia; suggestive link with non-Hodgkin lymphoma.	US EPA: 43% to 336% increase in leukemia in styrene-butadiene rubber workers, adjusting for styrene and benzene. IARC: Most recent update of the styrene-butadiene rubber worker cohort show no significant risk (IARC 2008).	US EPA: Group A; IARC: Group 1

Compound	Sources	Outcomes with strong evidence	Types of studies	Quantification (if possible)	Conclusion
Formaldehyde	US EPA 2010c; DRAFT IARC 2012; Checkoway et al. 2015	Nasal cancer Leukemia	Numerous cohort studies of occupationally exposed formaldehyde workers.	Nasopharyngeal cancer: RR = 4.14 for highest exposure (Hauptmann et al. 2004, as reported by US EPA 2010) All leukemia: RR=2.49, 95% CI: 1.13-5.49 for highest exposure) Chronic myeloid leukemia: RR=3.81, 95% CI: 0.36-40.44 for highest exposure (Checkoway et al. 2015)	US EPA - Category B1 (DRAFT); IARC - Group 1 - "Formaldehyde causes cancer of the nasopharynx and leukemia."
Vinyl chloride	US EPA 2000; IARC 2012; Ward et al. 2001; Mundt et al. 2000	Liver cancer	At least 14 cohort studies from the 1970s to 1990s of liver cancer in occupational workers, including 2 multicenter cohort studies (US and Europe)	RR=28.3, 95% CI: 12.8-62.3 for very high exposures (Ward et al. 2001) HR=6.0, 95% CI: 2.5-14.4 for exposures ≥ 20 years of exposure (Mundt et al. 2000)	US EPA: Category A ; IARC: Group 1 Mundt: "deaths from liver cancers have occurred in excess, due to the well documented association between VCM and angiosarcoma of the liver." Ward: "A strong relation is observed between cumulative VC exposure and occurrence of liver cancer."
Benzene	US EPA 2003; IARC 2012; Khalade et al. 2010	Leukemia	Numerous occupational benzene -exposed workers in the chemical industry, shoemaking, and oil refineries. Consistent excess risk of leukemia across studies	Pooled estimate (leukemia) 2.62 (95% CI, 1.57-4.39) for high exposures based on meta-analysis (Khalade et al. 2010)	US EPA - Category A; IARC - Group 1 "sufficient evidence" in humans for leukemia.
Trichloroethylene	US EPA 2011; IARC 2014	Kidney cancer	Numerous cohort and case -control studies with consistent evidence.	Pooled estimate (RR) = 1.58, 95% CI: 1.28, 1.96 based on meta-analysis of highest exposure group (US EPA 2011)	US EPA - Category CH; IARC - Group 2A

Compound	Sources	Outcomes with strong evidence	Types of studies	Quantification (if possible)	Conclusion
Epichlorohydrin	US EPA 1988b; IARC 1999	Inadequate data in humans. In animals, stomach and oral cavity cancers via oral and nasal tumors via inhalation exposure	4 cohort studies (including 3 nested case-control studies) found weak and inconsistent associations with lung cancer and central nervous system tumors with no dose-response (IARC 1999)	No risk estimates in humans available	US EPA - Category B2, IARC - Group 2A, "probably carcinogenic to humans," based on animal studies, the "known chemical reactivity of epichlorohydrin and its direct activity in a wide range of genetic tests."
Tetrachloroethene (Also tetrachloroethylene)	US EPA 2012; IARC 2014 Pesch et al. 2000 Radican et al. 2008 Seidler et al. 2007	Bladder cancer, non-Hodgkin lymphoma, multiple myeloma	**Bladder cancer: 10-14% increased risk** Five of the six occupational high quality studies (dry cleaner or laundry workers) **Non-Hodgkin lymphoma:** Five cohort high quality occupational studies **Multiple myeloma:** Little evidence from lower quality but larger cohort studies Some evidence with higher quality cohort and case control studies	**Bladder cancer:** RR = 1.8, 95% CI: 1.2, 2.7 high exposure (Pesch et al. 2000) **NHL:** RR = 3.4, 95% CI: 0.7, 17.3 for the highest exposure (Seidler et al. 2007) **Multiple myeloma:** Aircraft maintenance workers cohort RR men: 1.71, 95% CI: 0.42, 6.91 RR women: 7.84, 95% CI: 1.43, 43.1 (Radican et al. 2008)	US EPA - Category LH, IARC - Category 2A

CI: confidence interval
HR: hazard ratio
IARC: International Agency for Research on Cancer
NHL: Non-Hodgkin lymphoma
RR: relative risk
SIR: standardized incidence ratio
SMR: standardized mortality ratio
US EPA: United States Environmental Protection agency
VC: vinyl chloride
VCM: vinyl chloride monomer

APPENDIX C
MULTISTAGE WEIBULL MODELING OUTPUT

```
================================================================
          Multistage Weibull Model. (Version: 1.6.1;  Date: 11/24/2009)
          Solutions are obtained using donlp2   -intv, (c) by P. Spellucci
          Input  Data File:  FMLAd1In.(d)
          Tue May 02 10:15:41 2017
 ================================================================
 Female Mouse Lung C+ I Grouped Incidental Risk 1-stage MSW model
 ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
    The form of the probability function is:
   P[response]  = 1-EXP{ -(t - t_0)^c *
                 (beta_0+beta_1*dose^1)}
   The parameter betas are restricted to be positive
   Dependent variable = CLASS
   Independent variables = DOSE, TIME
 Total number of observations = 199
 Total number of records with missing values = 0
 Total number of parameters in model = 4
 Total number of specified parameters = 1
 Degree of polynomial = 1
   User specifies the following parameters:
          t_0    =         0
 Maximum number of iterations = 16
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-   008
                  Default Initial Parameter Values
                        c      =      2.65306
                        t_0    =            0   Specified
                        beta_0 = 3.87553e-007
                        beta_1 = 8.74531e-006
           Asymptotic Correlation Matrix of Parameter Estimates
           ( *** The model parameter(s)   -t_0
                 have been estimated at a boundary point, or have been specified by the user,
                 and do not appear in the correlation matrix )
                 c            beta_0       beta_1
    c                 1        -0.99           -1
    beta_0         -0.99            1         0.98
    beta_1           -1         0.98            1
Parameter Estimates
                                                        95.0% Wald Confidence Interval
       Variable        Estimate       Std. Err.       Lower Conf. Limit   Upper Conf. Limit
         c               2.7855        0.871309            1.07777             4.49324
         beta_0     2.09796e-007     8.59988e-007       -1.47575e-006       1.89534e-006
         beta_1     4.84999e-006     1.88357e-005       -3.20673e-005       4.17673e-005
                Log(likelihood)  # Param            AIC
   Fitted Model       -85.7218         3         177.444
```

Data Summary

DOSE	C	F	I	U	Total
0	46	0	4	0	50
0.74	21	0	28	0	49
1.2	16	0	34	0	50
1.6	8	0	42	0	50

Benchmark Dose Computation

Risk Response = Incidental
Risk Type = Extra
Specified effect = 0.01
Confidence level = 0.9
Time = 105
BMD = 0.00485752
BMDL = 0.00394674
BMDU = 0.00604099

```
================================================================
        Multistage Weibull Model. (Version: 1.6.1;  Date: 11/24/2009)
        Solutions are obtained using donlp2   -intv, (c) by P. Spellucci
        Input  Data File:  FMLAd1Io.(d)
        Tue May 02 09:56:18 2017
================================================================
Female Mouse Lung C+I+U Grouped  Incidental  Risk 1-stage  MSW model
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
   The form of the probability function is:
  P[response]  = 1-EXP{ -(t  -  t_0)^c *
                (beta_0+beta_1*dose^1)}
  The parameter  betas  are restricted  to be positive
  Dependent variable = CLASS
  Independent  variables  = DOSE,  TIME
Total number of observations = 200
Total  number  of records  with  missing  values  = 0
Total  number  of parameters  in model  = 4
Total number of specified parameters = 1
Degree of polynomial = 1
  User specifies the following parameters:
        t_0    =          0
Maximum number of iterations = 16
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-   008
                 Default Initial Parameter Values
                        c      =      2.70833
                        t_0    =            0   Specified
                        beta_0 = 2.99752e-007
                        beta_1 = 6.82409e-006
          Asymptotic Correlation Matrix of Parameter Estimates
          ( *** The model parameter(s)   -t_0
                have been estimated at a boundary point, or have been specified by the user,
                and do not appear in the correlation matrix )
                c             beta_0        beta_1
   c                 1         -0.98            -1
   beta_0         -0.98             1          0.98
   beta_1            -1          0.98             1
                                 Parameter  Estimates
                                                        95.0%  Wald  Confidence  Interval
      Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
        c              2.82393          0.86564            1.12731            4.52055
        beta_0     1.75446e-007      7.14572e-007       -1.22509e-006       1.57598e-006
        beta_1     4.07913e-006      1.57386e-005        -2.6768e-005       3.49262e-005
               Log(likelihood)  # Param            AIC
  Fitted Model        -85.8823         3         177.765
```

Data Summary

DOSE	CLASS C	F	I	U	Total
0	46	0	4	0	50
0.74	21	0	28	1	50
1.2	16	0	34	0	50
1.6	8	0	42	0	50

Benchmark Dose Computation

Risk Response	=	Incidental
Risk Type	=	Extra
Specified effect	=	0.01
Confidence level	=	0.9
Time	=	105
BMD	=	0.00482968
BMDL	=	0.00372838
BMDU	=	0.00600798

```
=====================================================================
         Multistage Weibull Model. (Version: 1.6.1;     Date: 11/24/2009)
         Solutions are obtained using donlp2   -intv, (c) by P. Spellucci
         Input  Data File:  FMLAd2In.(d)
         Tue May 02 09:56:30 2017
=====================================================================
Female  Mouse  Lung  C+I  Grouped  Incidental  Risk  2-stage  MSW model
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
   The form of the probability function is:
  P[response]  = 1-EXP{ -(t  -  t_0)^c *
               (beta_0+beta_1*d ose^1+beta_2*dose^2)}
  The parameter  betas  are restricted  to be positive
  Dependent variable = CLASS
  Independent  variables  = DOSE,  TIME
Total number of observations = 199
Total  number  of records  with  missing  values  = 0
Total  number  of parameters  in  model  = 5
Total number of specified parameters = 1
Degree of polynomial = 2
  User specifies the following parameters:
        t_0    =         0
Maximum number of iterations = 16
Relative Function Convergence has been set to: 1e-008
Parameter  Convergence has been set to: 1e-008
                 Default Initial Parameter Values
                       c      =      3.71429
                       t_0    =            0   Specified
                       beta_0 = 2.99856e-009
                       beta_1 =            0
                       beta_2 = 7.10296e-008
          Asymptotic Correlation Matrix of Parameter Estimates
          ( *** The model parameter(s)   -t_0       -beta_1
                have been estimated at a boundary point, or have been specified by the user,
                and do not appear in the correlation matrix )
                c             beta_0        beta_2
   c                 1         -0.99            -1
   beta_0         -0.99             1          0.99
   beta_2            -1          0.99             1
                                Parameter  Estimates
                                                        95.0% Wald  Confidence  Interval
      Variable          Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
        c               3.51729         0.955751            1.64405            5.39052
        beta_0     7.51777e-009      3.39426e-008       -5.90086e-008       7.40441e-008
        beta_1                0                NA
        beta_2     1.70594e-007      7.25361e-007       -1.25109e-006       1.59228e-006
```

NA - Indicates that this parameter has hit a
bound implied by some inequality constraint
and thus has n o standard error.

	Log(likelihood)	# Param	AIC
Fitted Model	-82.6686	4	173.337

Data Summary

	CLASS				
	C	F	I	U	Total
DOSE					
0	46	0	4	0	50
0.74	21	0	28	0	49
1.2	16	0	34	0	50
1.6	8	0	42	0	50

Benchmark Dose Computation

Risk Response = Incidental
Risk Type = Extra
Specified effect = 0.01
Confidence level = 0.9

Time = 105

BMD = 0.0676952
BMDL = 0.00685005
BMDU = 0.0770164

```
 ======================  ============================================
          Multistage Weibull Model. (Version: 1.6.1;  Date: 11/24/2009)
          Solutions are obtained using donlp2   -intv, (c) by P. Spellucci
          Input  Data  File:  FMLAd2Io.(d)
          Tue May 02 09:56:48 2017
 ========================================================================

 Female Mouse Lung C+I+U Grouped  Incidental  Risk  2-stage  MSW model

~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The  form  of  the  probability  function  is:
   P[response]  = 1-EXP{ -(t  -  t_0)^c *
                 (beta_0+beta_1*dose^1+beta_2*dose^2)}

   The  parameter  betas  are  restricted  to  be  positive

   Dependent variable = CLASS
   Independent  variables  = DOSE,  TIME

 Total number of observations = 200
 Total  number  of records  with  missing  values  = 0
 Total  number  of parameters  in model  = 5
 Total number of specified parameters = 1
 Degree of polynomial = 2



   User specifies the following parameters:
          t_0    =          0

 Maximum  number  of iterations = 16
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-   008


                  Default Initial Parameter Values
                         c      =      3.33333
                         t_0    =             0   Specified
                         beta_0 = 1.77269e-008
                         beta_1 =             0
                         beta_2 = 3.85864e-007


           Asymptotic Correlation Matrix of Parameter Estimates
           ( *** The  model  parameter(s)   -t_0       -beta_1
```

have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	c	beta_0	beta_2
c	1	-0.99	-1
beta_0	-0.99	1	0.99
beta_2	-1	0.99	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
c	3.53767	0.951903	1.67197	5.40336
beta_0	6.83164e-009	3.07193e-008	-5.33771e-008	6.70404e-008
beta_1	0	NA		
beta_2	1.55674e-007	6.59259e-007	-1.13645e-006	1.4478e-006

NA - Indicates that this parameter has hit a
bound implied by some inequality constraint
and thus has no standard error.

	Log(likelihood)	# Param	AIC
Fitted Model	-82.7393	4	173.479

Data Summary

DOSE	CLASS C	F	I	U	Total
0	46	0	4	0	50
0.74	21	0	28	1	50
1.2	16	0	34	0	50
1.6	8	0	42	0	50

Benchmark Dose Computation

Risk Response	=	Incidental
Risk Type	=	Extra
Specified effect	=	0.01
Confidence level	=	0.9
Time	=	105
BMD	=	0.0675827
BMDL	=	0.00695368
BMDU	=	0.0767564

```
 ================================================================
          Multistage Weibull Model. (Version: 1.6.1;  Date: 11/24/2009)
          Solutions are obtained using donlp2-intv, (c) by P. Spellucci
          Input Data File: FMLAd3In.(d)
          Tue May 02 09:57:04 2017

 ================================================================

 Female Mouse Lung C+I Grouped Incidental Risk 3-stage MSW model

~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:
   P[response] = 1-EXP{-(t - t_0)^c *
                 (beta_0+beta_1*dose^1+beta_2*dose^2+beta_3*dose^3)}

   The parameter betas are restricted to be positive

   Dependent variable = CLASS
   Independent variables = DOSE, TIME

 Total number of observations = 199
 Total number of records with missing values = 0
 Total number of parameters in model = 6
 Total number of specified parameters = 1
 Degree of polynomial = 3


   User specifies the following parameters:
          t_0    =          0

 Maximum number of iterations = 16
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-   008


                  Default Initial Parameter Values
                        c      =      3.51351
                        t_0    =            0   Specified
                        beta_0 = 7.69524e-009
                        beta_1 = 8.17936e-008
                        beta_2 =            0
                        beta_3 =  8.3075e-008
```

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -t_0 -beta_2
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	c	beta_0	beta_1	beta_3
c	1	-0.99	-0.99	-0.99
beta_0	-0.99	1	0.98	0.98
beta_1	-0.99	0.98	1	0.97
beta_3	-0.99	0.98	0.97	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
c	3.565	1.09332	1.42214	5.70787
beta_0	6.06284e-009	3.09921e-008	-5.46806e-008	6.68063e-008
beta_1	6.3958e-008	3.37242e-007	-5.97025e-007	7.24941e-007
beta_2	0	NA		
beta_3	6.69836e-008	3.08585e-007	-5.37832e-007	6.718e-007

NA - Indicates that this parameter has hit a
bound implied by some inequality constraint
and thus has no standard error.

	Log(likelihood)	# Param	AIC
Fitted Model	-82.6066	5	175.213

Data Summary

	CLASS C	F	I	U	Total
DOSE					
0	46	0	4	0	50
0.74	21	0	28	0	49
1.2	16	0	34	0	50
1.6	8	0	42	0	50

Benchmark Dose Computation

Risk Response = Incidental
Risk Type = Extra
Specified effect = 0.01
Confidence level = 0.9

Time = 105

BMD = 0.00978798
BMDL = 0.0052444
BMDU > 0.0783038

```
 ========================================    ==========================
          Multistage Weibull Model. (Version: 1.6.1;  Date: 11/24/2009)
          Solutions are obtained using donlp2   -intv, (c) by P. Spellucci
          Input  Data  File:  FMLAd3Io.(d)
          Tue May 02 09:58:50 2017
 ======================================================================

 Female Mouse Lung C+I+U Grouped  Incidental  Risk  3-stage  MSW model

~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The  form  of  the  probability  function  is:
   P[response]  = 1-EXP{ -(t  -  t_0)^c *
                 (beta_0+beta_1*dose^1+beta_2*dose^2+beta_3*dose^3)}

   The  parameter  betas  are  restricted  to  be  positive

   Dependent variable = CLASS
   Independent  variables  = DOSE,  TIME

 Total  number  of observations = 200
 Total  number  of records  with  missing  values  = 0
 Total  number  of parameters  in model  = 6
 Total number of specified parameters = 1
 Degree of polynomial = 3



   User specifies the following parameters:
          t_0    =          0

 Maximum number of iterations = 16
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-   008


                  Default Initial Parameter Values
                         c      =      3.02326
                         t_0    =            0   Specified
                         beta_0 =  7.4445e-008
                         beta_1 = 8.31425e-007
                         beta_2 =            0
                         beta_3 = 6.42289e-007


             Asymptotic Correlation Matrix of Parameter Estimates
```

(*** The model parameter(s) -t_0 -beta_2
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	c	beta_0	beta_1	beta_3
c	1	-0.99	-0.99	-0.99
beta_0	-0.99	1	0.98	0.98
beta_1	-0.99	0.98	1	0.97
beta_3	-0.99	0.98	0.97	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
c	3.59456	1.08684	1.4644	5.72473
beta_0	5.28712e-009	2.68702e-008	-4.73775e-008	5.79518e-008
beta_1	5.52071e-008	2.89531e-007	-5.12264e-007	6.22678e-007
beta_2	0	NA		
beta_3	5.93591e-008	2.72143e-007	-4.74031e-007	5.92749e-007

NA - Indicates that this parameter has hit a
bound implied by some inequality constraint
and thus has no standard error.

	Log(likelihood)	# Param	AIC
Fitted Model	-82.6739	5	175.348

Data Summary

DOSE	CLASS C	F	I	U	Total
0	46	0	4	0	50
0.74	21	0	28	1	50
1.2	16	0	34	0	50
1.6	8	0	42	0	50

Benchmark Dose Computation

Risk Response = Incidental
Risk Type = Extra
Specified effect = 0.01
Confidence level = 0.9
Time = 105
BMD = 0.00988202
BMDL = 0.0052649
BMDU > 0.0790561

APPENDIX D
ABOUT RAMBOLL ENVIRON

ABOUT RAMBOLL ENVIRON

A premier global consultancy, Ramboll Environ is trusted by clients to manage their most challenging environmental, health and social issues. We have earned a reputation for technical and scientific excellence, innovation and client service. Our independent science-first approach ensures that our strategic advice is objective and defensible. We apply integrated multidisciplinary services and tailor each solution to our client's specific needs and challenges.

At the end of 2014, ENVIRON joined forces with Ramboll, Northern Europe's leading engineering, design and management consultancy, to create a global practice called Ramboll Environment and Health. Together we provide an even higher level of service to our clients and address some of the most important issues facing our global community, including the environmental and health implications of urbanization, climate change and resource scarcity.

Ramboll Environ's network of experts includes more than 2,100 employees across 130 offices in 28 countries around the world. Clients will continue to benefit from our unique ability to bring clarity to issues at the intersection of science, business and policy.

APPENDIX E
EXPERT BIOGRAPHIES

P ROBINAN GENTRY

Principal/Operations Director – Gulf Coast

Dr. Robinan Gentry is a toxicologist with over 25 years of experience in toxicological issues relevant in the determination of the potential safety or risk associated with exposure to chemicals. Over her career, she has been a principal investigator or contributing author for numerous safety and risk assessments for both government and industry. She has worked as a government subcontractor in which she developed toxicological profiles for the US EPA IRIS program, ATSDR and FDA. Many assessments in which she has been involved has been to incorporate innovative quantitative approaches at that time (e.g., benchmark dose modelling, probabilistic assessments, PBPK modelling, in vitro to in vivo extrapolation, genomics data). She is a published author in the development of risk assessment methods, including Physiologically Based Pharmacokinetic (PBPK) models, and their application into both the cancer and non-cancer risk assessment process.

EXPERIENCE HIGHLIGHTS

Quantitative Risk Assessments

Managed numerous human health risk assessments and projects related to the development of criteria and other health effects documents, including application of benchmark modelling; conducted detailed analyses of guidance used in the determination of acute toxicity exposure levels and comparison of USEPA's and California's Proposition 65's risk assessment methods for multiple chemicals; quantified margin of exposures and cancer slope factor using existing kinetic and mechanism of action for multiple compounds.

Toxicological Reviews

Prepared toxicological reviews for USEPA's Office of Pesticide Programs and Program for Toxic Substances (OPPTS), FDA's Center for Food Safety and Nutrition, the Agency of Toxic Substances and Disease Registry (ATSDR), contributing author for development of Drinking Water Criteria Documents for several radionuclides and chloroform; development of weight-of-evidence evaluations and systemic reviews for multiple chemicals including formaldehyde, methyl salicylate and arsenic.

Pharmacokinetics and PBPK Modelling

Served as principal investigator or co-investigator for several PBPK modelling projects, including the development of models in multiple species for constituents such as coumarin, arsenic, acrylic acid and isopropanol.

CONTACT INFORMATION

P Robinan Gentry

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+1 (318) 3982083

Ramboll Environ
3107 Armand Street
Monroe, LA 71201
United States of America

CREDENTIALS

PhD, Toxicology, Utrecht University, The Netherlands

Diplomate. American Board of Toxicology, 2002; recertified, 2007, 2011

MS, Pharmacology & Toxicology, Northeast Louisiana University

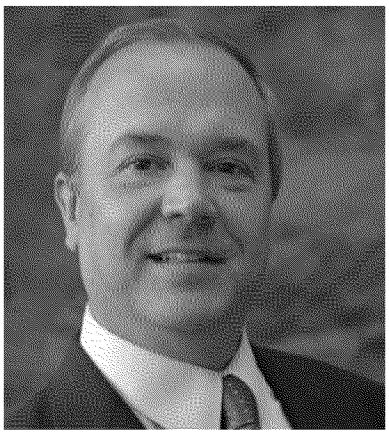
BS, Toxicology, Northeast Louisiana University

RAMBOLL ENVIRON

ENVIRONMENT & HEALTH

KENNETH A MUNDT

Principal



Dr. Kenneth Mundt is Health Sciences Practice Network Leader. He brings 30 years of experience in applying epidemiological concepts and methods to understand human health risks from environmental, occupational and consumer product exposures.

Dr. Mundt specializes in the pragmatic interpretation of epidemiological evidence in evaluating disease causation and supporting science-based regulation and decision-making.

Previously, Dr. Mundt served 11 years on the Graduate Faculty of the School of Public Health and Health Sciences, University of Massachusetts at Amherst. He received his PhD in Epidemiology at the University of North Carolina at Chapel Hill, and is a Fellow in the American College of Epidemiology.

EXPERIENCE HIGHLIGHTS

Epidemiological Studies
Managed multidisciplinary teams in designing, conducting and interpreting occupational epidemiological studies of workers involved in rubber, porcelain, chemical and steel industries, as well as military and other professionals.

Health Risks Evaluation and Communication
Responded to observed and perceived health problems related to occupational, environmental and consumer product exposures.

Teaching and Scholarship
Frequent participant in scientific meetings, training courses, and litigation proceedings. Consistent publication record.

Scientific Regulatory Support
Provided scientific evaluation and support to various regulatory and policy processes, including oral and written comments, statistical re-analysis of data from key studies, preparation of commentaries and technical communications, identification of new research opportunities, critical review and meta-analyses of epidemiological evidence, integration of scientific evidence from diverse lines of inquiry, organize and manage expert panels and topical symposia.

Critical Reviews and Syntheses
Comprehensively identified, systematically critically reviewed and synthesized the epidemiological literature on human health risks associated with numerous occupational, environmental and consumer product exposures.

CONTACT INFORMATION

Kenneth A Mundt

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Suite 2A
Amherst, 01002
United States of America

CREDENTIALS

PhD, Epidemiology
University of North Carolina

MS, Epidemiology
University of Massachusetts

MA, English
University of Virginia

AB, English
Dartmouth College

SONJA SAX

Senior Environmental Health Scientist

Dr. Sonja Sax is an environmental health scientist with over 15 years of exposure and health risk assessment experience. She has particular expertise in airborne gases and particles, and has performed indoor and outdoor air quality investigations, managed several large environmental projects, conducted critical evaluations of toxicology and epidemiology studies, and helped prepare technical and expert reports. Sonja has authored and co-authored several publications, presented her research and consulting work at various conferences and testified before scientific panels. Sonja earned an MS and doctorate in environmental health from the Harvard T.H. Chan School of Public Health, where she also served as a postdoctoral fellow.

EXPERIENCE HIGHLIGHTS

Critical Reviews and Syntheses
Conducted an extensive literature search on the toxicity and health effects of different chemical compounds including cobalt alloys found in dental materials, diesel exhaust, carbon black, welding fumes, particulate matter and sulfur dioxide.

Systematic Reviews
Conducted weight-of-evidence evaluation of cardiovascular and respiratory effects from exposures to ozone. Results were published in several peer-reviewed manuscripts.

Litigation Support
Contributed to the preparation of expert reports in litigation projects involving different chemical exposures (e.g., vinyl chloride, asbestos, carbon black, particulate matter, sulfur dioxide, and pesticides).

Exposure and Risk Assessment
For numerous projects prepared technical analyses on exposures and potential health effects associated with various pollutants (e.g., particulate matter, sulfur dioxide, nitrogen dioxide, arsenic, and pesticides). Exposure assessments included air dispersion modeling.

Regulatory Comments
Provided written and oral comments to the Clean Air Scientific Advisory Committee on exposure and health effects data and their bearing on US EPA's National Ambient Air Quality Standards for particulate matter and ozone.

Indoor Exposure and Risk Assessment
Conducted analyses of residential exposures to chemicals (e.g., formaldehyde from wood products, vapor intrusion of tetrachloroethylene, mercury from wallboard, and flame retardants from various indoor sources).

CONTACT INFORMATION

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United States of America

CREDENTIALS

ScD, Environmental Health Sciences
Harvard School of Public Health

MS, Environmental Health Management
Harvard School of Public Health

BA, Biological Chemistry
Wellesley College